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## A NEW OR LITTLE-KNOWN RICE DISEASE OCCURRING IN THE PHILIPPINES<sup>1</sup>

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SIX PLATES

In 1930, at Alabang, Rizal Province, Luzon, a fungous disease attacking a few young rice plants in isolated spots under lowland conditions causing foot-rot and wilt was first observed. The disease is not widespread in the locality and its occurrence is somewhat sporadic, but it kills rice plants at a certain stage (Plate 1, fig. 1) and might spread unduly if neglected and prove injurious to later plantings. The field manifestations of the disease, therefore, warrant some study. From the base of diseased stems, a fungus was isolated in pure culture whose cultural characters agree with *Fusarium* (Plate 1, fig. 3).

The investigation here reported is essentially preliminary in character, the main object being to assess the economic importance of the disease. Naturally the work calls for field observations. Simple field observations, however, would seem impossible in obtaining information as to the exact distribution of the disease over widely scattered areas in this country, and because it can so easily be confused with other rice maladies. Laboratory work is, therefore, essentially important for a correct diagnosis. Although the disease has been found so far only in Rizal Province, it seems probable that it will eventually be

<sup>1</sup> Experiments performed while the writer was connected with the Bureau of Science, Manila.

encountered elsewhere. At present, however, it is impossible to make an accurate estimate of the damage done, hence, it may be considered of minor importance because a wide survey is wanting and also because of the fact that its incidence and parasitic nature are just beginning to be recognized in this country. Further studies which suggest themselves may be undertaken to find out definitely the exact taxonomic position and bionomics of the causal fungus. A study of the relative susceptibility of rice varieties to the disease will undoubtedly be of great value as well as its response to other means of control.

In order to determine the distribution and economic importance of the disease in the rice regions, the help of those who are in touch with the field situation is earnestly requested. This brief note, it is hoped, will familiarize field workers with the disease so that it may be reported at once when it is encountered. Specimen plants will be greatly appreciated.

#### OCCURRENCE OF APPARENTLY SIMILAR DISEASES IN OTHER COUNTRIES

*Fusarium*-type of diseases (fusariose) have been reported as attacking rice in one way or another in other countries, the species of which have not been definitely ascertained. Of late, a new *Fusarium* disease has been reported as causing considerable injury to rice in Madras by Sundararaman<sup>(11)</sup>, the attack consisting of foot-rot and wilt. A foot-rot disease of rice caused by an unidentified species of *Fusarium*, exhibiting many things in common with the Japanese "bakanae" mentioned here, is also being studied in Madras by Thomas.<sup>(13, 14)</sup> McRae<sup>(6)</sup> also reports a *Fusarium* disease of rice in India which can kill rice seedlings and induce barrenness in plants which overcome the effects of the disease. A similar type of disease has been recorded<sup>(2)</sup> in Japan, where it is called "bakanae" by Seto,<sup>(10)</sup> and Ito and Kimura,<sup>(4)</sup> probably because of the growth-stimulating influence of the fungus on the rice plant; and in British Guiana by Martyn<sup>(5)</sup> who calls it "man rice," because of the tall and spindly appearance of the affected plants. In the latter country, the disease was reported to be not so serious as in Madras, which seems to be true also in the Philippines. In some respects, the Philippine *Fusarium* disease of rice corresponds with the description of a somewhat similar disease reported by Sundararaman,<sup>(11)</sup> Seto,<sup>(10)</sup> and other workers, but whether or not the disease found in the Philippines is identical with those

reported in other rice-producing countries cannot be definitely ascertained at present. Furthermore, it is almost impossible to overcome the insuperable difficulty of gaining access to literature on the seemingly same disease in other countries, particularly that in Japanese, aside from the fact that most of the available accounts are rather brief and, therefore, inadequate for comparative purposes.

A search through Philippine literature failed to show any description or semblance of the disease in question. There seems no doubt, however, that the disease in the Philippines is caused by a species of *Fusarium*, and, so far as known, this peculiar disease condition is not known to have occurred heretofore on rice in the Philippines and has not been previously reported.

#### SYMPTOMS AND SIGNS

From a distance, the affected plants are likely to be mistaken by the unpracticed eye for rice plants ravaged by an insect stem borer, the leaves being discolored, curled, and drooping in later stages of infection. The characteristic effects are almost similar except that the plants affected by the fungous disease may be occasionally impeded in growth, if infection takes place at an early stage, or they may be disproportionately tall and slender, as generally happens, and occasionally show fewer tillers. On closer examination, by pulling some newly diseased individuals, it was noted they do not break easily at the base (Plate 1, fig. 1) and by examining diseased parts under the microscope it was found that they are affected by a fungus capable of causing wilting, the hyaline septate hyphae invading the tissues.

Externally the signs of the disease simulate also somewhat the withered condition encountered with other members of the grass (Gramineæ) family. In an early stage of the disease, some plants showed evident signs of wilting. In some plants only one or two shoots are affected, or the plants may be either partially or entirely wilted, the rest being apparently unaffected. The leaves and stems die out gradually, losing their normal color, and the presence of masses of white or pale salmon-colored, slimy fungous growths, extending upwards and down to the ground level, consisting of a thick network of mycelium and millions of conidia pasting the sheaths together, may be seen later on the outside of the more or less disintegrated stem, especially on the exposed portions of the lower nodes. Sporodochial masses of the causative fungus are sometimes visible, lining the

margins of the basal portions of the sheaths (Plate 1, fig. 2). The basal portion of the stem under water is somewhat darkened by decay of the affected tissues. To all appearances the underground roots are found to be normal (Plate 1, fig. 1).

By splitting the infected culm into halves it will be observed that the affected tissues are discolored, and accumulations of the fungus become visible, especially at the nodes which are usually browned, and the fungous hyphæ pervade all parts. The infection presumably starts from the base of the plant, the fungus being generally a soil inhabitant, and finds its way to the heart of the plant which offers a congenial habitat for the organism, causing rotting of the top as a consequence of infection.

#### THE CAUSAL FUNGUS AND ITS RELATION TO RICE FUSARIUM-TYPES OF DISEASES FOUND ELSEWHERE

Isolations of the fungus were made from young and old cases of the disease by planting sterile fragments of the infected tissues removed with a sterile scalpel from the basal parts of the plant on corn meal or steamed rice. Spore dilutions were also made on cooled poured plates of potato dextrose agar (Plate 1, fig. 3), to each of which a drop of 50 per cent lactic acid had been added and both methods yielded in the majority of cases similar colonies which were typical of *Fusaria*.

Microscopic examinations of the pure cultures of the organism proved beyond doubt that the causal fungus constantly associated with the disease belongs to the genus *Fusarium*, the exact identity of which is still undetermined, although certain morphological characters as well as physiological seem to agree closely with those of section *Liseola* and of the *Moniliforme* group,<sup>(9)</sup> especially as to shape, size, and number of conidia, apparent absence of chlamydospores, and in growth behavior in different media.

Briefly, from the morphological and physiological standpoints, the Philippine rice *Fusarium* bears a quite close relationship to the *Moniliforme* group, producing profusely unicellular thin-walled, hyaline, 0-septate conidia or microconidia (Plate 2, fig. 1) in chains, ovoid to fusoid; formed in white to deep olive-buff<sup>2</sup> aërial, thin, fluffy mycelium on green bean pod culture. The macroconidia (Plate 2, fig. 2) are abundantly produced. They are hyaline, slender, sickle-shaped although some are almost

<sup>2</sup> The colors indicated here are those of Robert Ridgway's Color Standards and Color Nomenclature. Washington, D. C. (1912).

straight, attenuate, pedicellate, forming sporodochia in nature and on green bean pod culture, and pionnotal masses, generally on the vacant surfaces of the potato tuber-plug medium. The pionnates are usually light pinkish cinnamon to pinkish cinnamon on potato tuber plug. There are 1- to 5-septate conidia (Plate 2, fig. 2) but they are predominately 1- and 3-septate. They also vary in size and shape. Two-, 4-, and 5-septate spores are rare, especially the last. Conidia mounted in sterile water, with the edges of the cover glass waxed, germinated readily (Plate 2, figs. 3 and 4) producing swellings of the cells of the septate conidia and constrictions at the septa. The conidio-phores are highly branched (Plate 2, fig. 5). From examinations made under the microscope, no chlamydospores were seen. The stromatic layers were dusky slate blue on potato glucose agar and lavender violet on potato tuber-plug culture.

Very little is known concerning the causal fungus and reports on the subject are rather conflicting. In British Guiana, however, Martyn(5) cultured from rice plants manifesting apparently similar symptoms, a fungus among others, which he ascribed to *Fusarium moniliforme* Sheldon [= *Gibberella moniliformis* (Sheld.) Wineland]. Hemmi and Seto(2) obtained a fungus from the basal parts of diseased rice in Japan producing infection types similar to that of the Philippine rice *Fusarium* which they attributed to a species of *Fusarium*, the ascigerous stage of which was described by Sawada in 1917 as *Lisea fujikuroi*. In the studies of Hemmi, Seto, and Ikeya,(3) on the "bakanae" disease of the rice plant, they demonstrated that both *Lisea* (*Gibberella*) *fujikuroi* and *G. saubinetii* are capable of infecting rice plants during the blooming period, producing identical symptoms. From infected seeds of healthy aspect, the rice plants produced exhibited the stimulatory and inhibitory effect of *G. fujikuroi* and *G. saubinetii*, respectively. On the other hand, the Japanese investigators, Ito and Kimura,(4) believed that the fungus they found on a similar disease of rice, is identical with, or possibly a variant of, *G. moniliformis* and the binomial *G. fujikuroi* (Sawada) S. Ito n. comb. has been proposed.

In determining the relationships between *Lisea fujikuroi*, the causal fungus of the "bakanae" disease of rice and *Fusarium moniliforme* var. *maius*<sup>3</sup> Wr. and Rg., the causal agent of the pokkah-boeng of sugar cane, Nisikado(7) found that these or-

<sup>3</sup> The Japanese authors wrote the variety name *majus*.

ganisms when inoculated into rice and maize seedlings produced the typical abnormal development characterizing the "bakanae" disease of rice. In view of this pathological proof, Nisikado supports the opinion of Wollenweber that *F. moniliforme* var. *maius* is the conidial form of *L. fujikuroi* and suggests that the name of the latter fungus be changed to *Gibberella fujikuroi* (Saw.) Wr. Based on the recent findings of Nisikado(7), therefore, the new combination proposed by Ito (and Kimura?)(4) and that of Wollenweber as cited by Nisikado(7) are one and synonymous. As the papers of these authors were both published in 1931, the writer is in a quandary as to which one has priority, or which one should be retained. This finding of Nisikado in experiments conducted previously in Biologische Reichsanstalt, Berlin-Dahlem, was, however, corrected later by Nisikado and Matsumoto(8) as erroneous since the Mexican strain of *F. moniliforme* var. *maius* revealed no virulent parasitic potentiality to rice or maize. In passing, it is thus apparent that there is considerable chaos as to the nomenclature of the *Fusarium*-type of disease of the rice plant.

#### INOCULATION EXPERIMENTS

Apparently two different strains of *Fusarium* were obtained from the separate isolations made from different individual cases, and pure cultures of these were used in inoculations made simultaneously on the stems of Magcumpol rice variety by inserting bits of the fungus between two outermost leaf sheaths, sterilized on the surface, above the level of irrigation water. As a result of the tests, only one of the organisms proved pathogenic as shown in Table 1.

TABLE 1.—Preliminary results of tests for the parasitic potentiality of the organisms isolated.

| Pots.      | Organism A.      |   |   |   |                 |                     | Organism B.      |    |    |    |                 |                    |
|------------|------------------|---|---|---|-----------------|---------------------|------------------|----|----|----|-----------------|--------------------|
|            | Culms per plant. |   |   |   | Culms infected. | Per cent infection. | Culms per plant. |    |    |    | Culms infected. | Percent infection. |
|            |                  |   |   |   |                 |                     |                  |    |    |    |                 |                    |
| 1-----     | 6                | 7 | 8 | 5 | 6               | 23.0                | 11               | 8  | 11 | 10 | 0               | 0                  |
| 2-----     | 8                | 4 | 4 | 7 | 23              | 100.0               | 10               | 8  | 5  | 5  | 0               | 0                  |
| 3-----     | 7                | 6 | 1 | 7 | 3               | 13.3                | 7                | 6  | 9  | 11 | 0               | 0                  |
| Check----- | 4                | 7 | 8 | 6 | 0               | 0                   | 7                | 11 | 6  | 11 | 0               | 0                  |

The foregoing figures show the results in preliminary tests of the parasitic potentiality of the two strains of *Fusarium* isolated and cultivated in pure culture. It will be seen that only organism A produced successful infection (Plate 3, fig. 1) on

healthy rice plants the incubation period lasting for several days, while the other had negative results. Although these tests were found satisfactory, the quantity was insufficient and further experiments using organism A will be found elsewhere. Organism B produced only brown localized infection confined to the outer sheaths and the infected areas had not advanced far enough to cause any death of the individual culms inoculated. With organism A brown water-soaked lesions were produced, at first, which extended lengthwise both above and below the point of inoculation to as much as 15 centimeters upwards, and in some cases the brown discoloration reaching the base of the youngest leaf scaling along the margin of the leaf sheath. The older, more or less withered or moribund sheaths were more readily attacked than the younger ones, serving as bases of operations, the leaves drying up one after another.

The infected stems gradually became straw colored, and a powdery incrustation of the fungus consisting of innumerable spores and mycelia developed profusely on the aërial surface (Plate 3, figs. 2 and 3). The infected culms showed the fungus growth externally by removing the sheaths carefully, and also internally, especially at the nodes. Spores thus formed may be carried by the wind, rain, or other agencies. In an advanced stage of infection, the inner tissues of the stem were consumed by the fungus and only the thin outer wall (somewhat silicious epidermis) at the lower internodes remained intact. The stem was somewhat shriveled and dried and very light in weight. The infected stems were so greatly disorganized that they could be easily severed at the nodes where an accumulation of the fungus could be seen. The sheaths covering the stems were pasted together by mycelial strands.

INFECTION BY INSERTION OF THE FUNGUS, SPRAYING WITH SPORES,  
AND THROUGH INFECTED SOIL

In the following season, using apparently disease-free and sterilized seeds of the same pure line of rice used in the preceding experiment, supplementary series of inoculations were subsequently conducted on rice plants grown in big cement pots in the open, using the *Fusarium* which was recovered in the first inoculation trials. Various methods of inoculation, observing the usual aseptic precaution, and reproducing as nearly as possible the conditions that actually obtain in nature, were employed, such as placing bits of the fungus between two healthy green sheaths along the margin; spraying the top of the plant

with spores suspended in sterile water with the help of an atomizer; and planting rice in soils in which *Fusarium*-infected rice straw was allowed to rot in moist soil. Control plants were given the same treatment except that the inoculum was omitted. With the exception of soil infection, all soils used were previously moistened and sterilized in an autoclave for three consecutive hours at 15 pounds pressure. Table 2 gives the details with the results of inoculation obtained from these trials.

TABLE 2.—Artificial infection of rice plants using different methods of introducing the fungus.

| Number of plants. | Stem inoculation. |               |                         |                             |        | Spore suspension. |               |                         |                             |        |
|-------------------|-------------------|---------------|-------------------------|-----------------------------|--------|-------------------|---------------|-------------------------|-----------------------------|--------|
|                   | Culms.            | Bear-<br>ing. | Dis-<br>eased<br>culms. | Per cent<br>mortal-<br>ity. | Yield. | Culms.            | Bear-<br>ing. | Dis-<br>eased<br>culms. | Per cent<br>mortal-<br>ity. | Yield. |
| 1-----            | 8                 | 0             | 8                       | 100                         | 0      | 11                | 10            | 3                       | 27                          | 36.6   |
| 2-----            | 6                 | 4             | 3                       | 50                          | 12.6   | 10                | 7             | 3                       | 30                          | 21.2   |
| 3-----            | 7                 | 4             | 3                       | 43                          | 11.5   | 12                | 8             | 3                       | 25                          | 26.2   |
| 4-----            | 8                 | 5             | 3                       | 37                          | 17.9   | 12                | 9             | 3                       | 25                          | 38.0   |
| 5-----            | 9                 | 0             | 9                       | 100                         | 0      | 7                 | 1             | 2                       | 28                          | 0      |
| 6-----            | 12                | 6             | 6                       | 50                          | 27.9   | 8                 | 4             | 4                       | 50                          | 6.8    |
| 7-----            | 7                 | 4             | 4                       | 57                          | 12.5   | 4                 | 4             | 0                       | 0                           | 7.5    |
| 8-----            | 13                | 7             | 6                       | 46                          | 22.4   | 6                 | 3             | 2                       | 33                          | 6.7    |
| 9-----            | 9                 | 3             | 3                       | 33                          | 0      | 6                 | 0             | 2                       | 33                          | 0      |
| 10-----           | 8                 | 1             | 3                       | 37                          | 0      | 7                 | 0             | 1                       | 14                          | 0      |
| 11-----           | 9                 | 0             | 9                       | 100                         | 0      | 6                 | 0             | 1                       | 6                           | 0      |
| 12-----           | 10                | 4             | 10                      | 100                         | 0.8    | 9                 | 0             | 0                       | 0                           | 0      |
| Total---          | 106               | 38            | 67                      | 753                         | 105.6  | 98                | 46            | 24                      | 271                         | 143.0  |
| Average---        |                   |               |                         | 62.7                        | 8.8    |                   |               |                         | 22.5                        | 11.8   |

| Number of plants. | Infected soil. |               |                         |                             |        | Control. |               |               |                      |        |
|-------------------|----------------|---------------|-------------------------|-----------------------------|--------|----------|---------------|---------------|----------------------|--------|
|                   | Culms.         | Bear-<br>ing. | Dis-<br>eased<br>culms. | Per cent<br>mortal-<br>ity. | Yield. | Culms.   | Bear-<br>ing. | Dis-<br>eased | Per cent<br>bearing. | Yield. |
| 1-----            | 3              | 3             | 1                       | 33                          | 6.6    | 8        | 8             | 0             | 100                  | 22.0   |
| 2-----            | 2              | 2             | 0                       | 0                           | 4.8    | 7        | 7             | 0             | 100                  | 21.8   |
| 3-----            | 2              | 2             | 0                       | 0                           | 4.8    | 7        | 7             | 0             | 100                  | 23.7   |
| 4-----            | 1              | 1             | 0                       | 0                           | 2.8    | 6        | 6             | 0             | 100                  | 25.6   |
| 5-----            | 5              | 4             | 1                       | 20                          | 11.1   | 6        | 6             | 0             | 100                  | 29.0   |
| 6-----            | 5              | 4             | 1                       | 20                          | 8.3    | 6        | 6             | 0             | 100                  | 16.5   |
| 7-----            | 4              | 4             | 0                       | 0                           | 6.8    | 9        | 9             | 0             | 100                  | 35.7   |
| 8-----            | 5              | 4             | 1                       | 20                          | 8.2    | 8        | 8             | 0             | 100                  | 42.0   |
| 9-----            | 3              | 2             | 1                       | 33                          | 4.2    | 8        | 8             | 0             | 100                  | 35.2   |
| 10-----           | 3              | 2             | 0                       | 0                           | 6.3    | 6        | 6             | 0             | 100                  | 26.5   |
| 11-----           | 3              | 3             | 1                       | 33                          | 6.0    | 7        | 7             | 0             | 100                  | 25.7   |
| 12-----           | 2              | 2             | 0                       | 0                           | 4.3    | 6        | 6             | 0             | 100                  | 23.9   |
| Total---          | 38             | 33            | 6                       | 159                         | 74.2   | 84       | 84            | 0             | 100                  | 327.6  |
| Average---        |                |               |                         | 13.2                        | 6.3    |          |               |               | 100                  | 25.6   |

## RESULTS AND DISCUSSION

The data presented in Table 2 show clearly that stem inoculation proved the most effective of all the methods tried, causing an average mortality of 62.7 per cent. Spore suspension caused 22.5 per cent infection, and soil infection, 13.2 per cent. The controls remained healthy. In determining the percentages of infection, the number of plants infected were not used as a basis as these would not represent the actual damage caused by the disease, instead, the number of diseased culms were counted.

Spraying with spores was not so effective as direct stem inoculation probably because some of the spores adhering to the young leaves, in some cases, were outgrown by the rapid production of new leaves, or else they might have overcome the effects of the disease and the plants continued to develop normally. This method, however, proved somewhat more advantageous than soil inoculation because of exposure of the plants also to other infection caused perhaps by the dripping of droplets loaded with spores down the stems to the surface of irrigation water and probably to the soil. While spore and soil infections were of small consequence in these tests, it is believed, nevertheless, that the spread of the disease may be also due either to infection through irrigation water or through the soil when the fungus comes in contact with the plant under favorable weather and soil conditions, gaining entrance directly through the epidermis.

The immediate effect of the disease is the appearance of brown lesions around the point of inoculation, followed by yellowing of the outer leaves and wilting of the darkened youngest leaf (Plate 4, figs. 2 and 3). The outer leaves fold later and turn yellow, but under conditions that become favorable for the fungus the entire plant may be totally wilted and showed browning of the fibrovascular region. In some cases one or more culms were affected in a clump while the rest remained apparently healthy (Plate 4, fig. 2). Few cases only showed slight wilting and a few of the leaves yellowed. The fungus made much more conspicuous rapid progress upward, extending for several inches than downward, due probably to the hardness of the tissues at the basal portion of the plant. Nevertheless, although infection was very much deferred and the growth of some of the plants was stimulated, giving the appearance of an admixture of seeds of varying duration, it proved fatal to

some of the inoculated plants, the neighboring culms withering gradually until the entire stool was completely affected and killed. This acceleration to abnormal development (Plate 4, fig. 1, right pot), is believed by Hemmi and Seto<sup>(2)</sup> to be correlated with the toxic secretions of the causal fungus. Besides the mortality resulting from the attack, some of the surviving plants developed poor growth, few tillers, and as a consequence, short distorted or semisterile heads were occasionally produced. The heads were not well filled, and threshing revealed a lighter yield than would normally be expected. By removing the sheaths partly and by splitting the culms longitudinally, the fungus could be clearly seen on the surface and in the hollow of the internodes, especially at the nodes (Plate 5, figs. 1 and 2). Under favorable conditions quicker and better results would probably be obtained. A prolonged warm moist weather seems to favor the disease greatly.

Artificially induced disease substantiates field observations that occasionally wilting takes place before the plants succumb to the disease, the youngest leaf being the first to fold (Plate 4, fig. 2). In general the inoculated plants yielded a comparatively lesser amount of grains than the normal plants as a result of the disease. The average yield of the control plants was 25.6 grams while the infected plants yielded in the order given in Table 2, 8.8, 11.8, and 6.3 grams, respectively, or a decrease of 65.6, 53.9, and 75.3 per cent. As these are the outcome of artificially induced disease, the percentages are much greater than what normally obtain under field conditions. It is believed, however, that the tests detailed sufficiently established the pathogenicity of the fungus to rice, although not carried out on a very large scale.

#### INFECTION OF RICE PLANTS THROUGH THE ROOTS

Besides the methods used in the previous tests, an attempt was made to reproduce the disease by placing hyphae and spores of the fungus obtained from pure cultures near the living roots, without inflicting any injury, of rice grown in a greenhouse in root study boxes (Plate 6), 50 centimeters long, 28 centimeters wide, and 40 centimeters high, patterned after Dean.<sup>(1)</sup> The inoculations and observations were greatly facilitated by tilting the boxes so that the roots would grow next to one of the glass sides, protected by wooden shutter, by geotropic response. The results of these trials are shown in Table 3.

TABLE 3.—*Root inoculations with the rice Fusarium.*

| Number of plants. | Culms.     |          |           |
|-------------------|------------|----------|-----------|
|                   | Per plant. | Bearing. | Diseased. |
| <i>Inoculated</i> |            |          |           |
| 1.....            | 6          | 6        | 0         |
| 2.....            | 8          | 7        | 0         |
| 3.....            | 7          | 7        | 0         |
| 4.....            | 6          | 6        | 0         |
| 5.....            | 3          | 3        | 0         |
| 6.....            | 4          | 4        | 0         |
| <i>Controls</i>   |            |          |           |
| 7.....            | 4          | 4        | 0         |
| 8.....            | 6          | 6        | 0         |

Table 3 shows that the application of the fungus made no difference to subsequent growth. No infection developed in plants infected through the actively growing roots and the plants grew normally to maturity (Plate 6), although not as vigorous as those grown in the open. As the plants tested were few on account of limited facilities, it would seem that this point needs further elucidation before arriving at any conclusion.

#### DISSEMINATION

There are several agencies whereby the fungus may be distributed in the field. Infected stems if allowed to remain in the field after harvest become an important source of infection as the mycelium and spores of the fungus are practically unrestrained in propagating and increasing their numbers. The fungus is capable of living saprophitically in the soil on dead tissues and may attack the host plant the following season. Driving rains or irrigation water may carry the disease to lower level lands. Heavy winds undoubtedly also play an important rôle in scattering the causal fungus. Lodging plants and thick planting are also factors in spreading the disease from one plant to another. Dissemination of the fungus may also take place by mechanical means, by insects, man, animals, tools, and implements. There is very little chance of the disease being dispersed by diseased seeds, however, as diseased plants were generally killed before the heads appeared. There are no definite data to show that the disease is transmitted by seeds, but it seems reasonable to believe that the disease may be seed-borne.

## TRANSMISSION OF THE DISEASE THROUGH SEEDS

It was suspected that the fungus might be carried by the seeds. An attempt was therefore made to grow rice from seeds obtained from partially infected plants; that is, from apparently normal culms in infected clumps. The resulting plants developed normally. Although the tests were not sufficiently extensive to provide convincing evidence that the fungus is never carried in this manner, there is ground for believing that the fungus is probably sparingly present; but seeds obtained from partially infected plants should nevertheless be looked upon with suspicion and regarded as unsuitable for propagation purposes.

## VARIETAL SUSCEPTIBILITY

Plants grown under field conditions, based on microscopical symptoms of the disease, seem to indicate that individual resistance exists and that the reaction to the disease differs among varieties. While the writer has made no statistical observations on the incidence of the disease, the relative amount of disease on the varieties was estimated in paddies which had been previously fertilized with calcium nitrate. Just what constituent is responsible for the occurrence is not known, but it is probable that nitrogenous fertilizer is a predisposing factor as this would render the stems more succulent. Magcumpol and Inadhica showed a greater amount of disease than the other varieties which were grown in long, narrow plots only 40 centimeters apart. Some varieties appeared to be very little affected, if at all. Kinatuday showed very slight susceptibility, while Macan Biñan, Matayosa, Visaya, Guinagang Str. 1 and Gallano were practically unaffected by the disease. Random observations, however, are not very satisfactory as a basis for judging the resistance of different varieties to a given crop. The differences in the observations may be due to such factors as differences in the distribution of the natural inoculum, or to differences in degree of maturation of the varieties, or to variations in the response of the host strains. There seems, however, great promise of selection towards this direction.

In view of the above discrepancies, artificial infections on a few individuals of important varieties were undertaken in 1932, in the open, by inserting spores and hyphæ obtained from young cultures of the rice *Fusarium* between green leaf sheaths above the surface of irrigation water. The seeds used were previously treated for seed-borne diseases before sowing them in sterilized

soil. The seedlings were later transplanted in comparatively large cement pots. Plants held as controls were placed some distance away to obviate the possibility of contamination. The varieties of rice used in this test and the results obtained are recorded in Table 4.

TABLE 4.—Relative susceptibility of rice varieties to infection by rice *Fusarium*.

| Variety.          | Plants. | Inoculated.  |                 |               | Control. |              |                 |               |
|-------------------|---------|--------------|-----------------|---------------|----------|--------------|-----------------|---------------|
|                   |         | Total culms. | Culms infected. | Infection.    | Plants.  | Total culms. | Culms infected. | Infection.    |
|                   |         |              |                 | <i>P. ct.</i> |          |              |                 | <i>P. ct.</i> |
| Guinangang Str. 1 | 15      | 69           | 1               | 1.4           | 5        | 38           | 0               | 0             |
| Apostol           | 15      | 62           | 0               | 0             | 5        | 25           | 0               | 0             |
| Magcumpol         | 15      | 51           | 10              | 19.6          | 5        | 16           | 0               | 0             |
| Macan I           | 15      | 61           | 7               | 11.4          | 5        | 18           | 0               | 0             |
| Khao Bai Sri      | 15      | 61           | 3               | 4.9           | 5        | 19           | 0               | 0             |
| Inadhika          | 15      | 57           | 8               | 14.0          | 5        | 21           | 0               | 0             |
| Ramay             | 15      | 67           | 7               | 11.4          | 5        | 23           | 0               | 0             |
| Elonelon          | 15      | 55           | 4               | 7.2           | 5        | 30           | 0               | 0             |

From the above experiment some idea was further made apparent that the degree of susceptibility varied among rice varieties. In this table, it is shown that a comparatively small number of infections was observed even on the known susceptible varieties and the proportion of diseased to healthy plants was low as shown by the final records taken, indicating that the fungus was not probably a vigorous parasite, or that the environmental conditions then prevalent were not very conducive to its development. The results tend also to show that practically all varieties used are liable to the attack of the disease although this may not be true with other varieties. Apostol was found to possess greater resistance than any other of the varieties tested. Guinangang Str. 1 also showed considerable resistance and to some extent Khao Bai Sri, while Magcumpol, Inadhika, Macan I, Ramay, and Elonelon proved quite susceptible. This experiment, although not extensive, confirms somewhat the field observations. Some of these varieties are not of wide commercial importance and at present it does not seem necessary to resort to the use of less commercially-grown varieties, even if they show slightly greater resistance. Although the best prospect of practical control seems to lie in the discovery of resistant varieties, further observations and experiments along this line will undoubtedly be of value.

Sundararaman, (12) in India, reported wide variations in the susceptibility of rice varieties to *Fusarium* causing foot-rot of rice. In field tests made in 1931, certain varieties showed great susceptibility, while E. B. 24 was totally immune. Kistnakatukulu, Akkulu, Nallarlu, Atragada, and Vankisannam were highly resistant. With the exception of Nallarlu, which was omitted by Thomas in the 1932 tests, (14) these varieties were found later to be not so resistant as they were thought to be. The same author, (14) working in Coimbatore, India, tested forty-one varieties, but found none was absolutely immune to the disease. Wataribune, Aryan, and G. E. B. 24 proved highly resistant.

#### CONTROL MEASURES

Although the specific name of the fungus is not definitely known, it is doubtful if the means of control here formulated would undergo radical changes. To prevent the perpetuation of this disease, it is important that the farmers should be able to recognize it and, consequently, the sources of infection should be well guarded against and destroyed. As a result of this investigation, the following control procedures may be suggested:

Careful roguing of diseased plants as soon as detected in fields of low percentage of infection would be beneficial in reducing the primary source of infection. Great care should be exercised so as not to scatter the fungus about the field.

In diseased fields it seems expedient to burn the stubble after harvest or as soon as practicable as it is a source of major importance in natural infection; and plow the soil afterwards taking care that the furrows slice is turned over so as to partly starve the fungus present in the soil surface.

Rice being an annual crop, serious infestation may be avoided by rotation with widely different hosts. But as it is not known to what extent the fungus can survive a saprophytic life in the soil, a long time should elapse before the infected field is planted to rice again, judging from the knowledge of other *Fusarium* diseases. At least we are sure that by so doing the food supply is regulated. The growing, however, of resistant varieties should be preferred to carrying on a long period of rotation to starve out the fungus.

Soil from a *Fusarium*-infected field, irrigation water, or fragments of infected plant tissues should not be transferred from a diseased to a disease-free area. Tools and implements, work animals, men, and other mechanical means that might perhaps

carry the disease from one place to another should be guarded against.

It is not definitely known whether or not this disease can be spread by seeds, but it seems advisable to avoid such a possibility. Use only carefully selected seeds, preferably those obtained from disease-free fields, for propagation purposes.

#### SUMMARY

A *Fusarium* disease of rice, hitherto unrecognized and probably confused with other rice maladies, producing two closely associated pathological conditions—foot-rot and wilt—is here described for the first time. It is known to occur thus far in Rizal Province, and because of its limited distribution it is not considered of great economic importance at present.

The initial characteristic symptom of the disease is the yellowing of the outer leaves, then the younger leaves wilt, and later the affected plant finally dies. Frequently one or more tillers in a stool die, or the leaves are only yellowed, while the rest remains healthy. The vascular regions of infected plants are browned, and by splitting the stem in advanced stage of infection, a bloom of the fungus can be seen in the hollow of the stem accumulating more densely around the nodal region. The fungous growth is also evident outside the stem.

The disease is caused by a *Fusarium*, the specific identity of which remains to be determined. In other countries, closely related types of rice diseases have been described which are perhaps similar, if not identical, with that of the Philippines.

The pathogenicity of the causal fungus has been definitely ascertained, the degree of infection varying with the method of infection employed and with changes of weather conditions. That the disease produces foot-rot and wilt seems to have been demonstrated. The inference drawn from inoculation trials was that this rice *Fusarium* disease works rather slowly, and seems to be of little pathological significance at present.

The disease may be checked in a large measure by roguing and burning diseased plants in low percentage of infection as soon as they are noticed.

After harvest, or as soon as practicable, stubble that harbors the fungus should be burned completely so as to prevent the multiplication and recurrence of the disease.

Only carefully selected and cleaned seeds, preferably from a disease-free field, should be used for planting.

As the fungus seems to be persistent in the soil a long period of rotation will be necessary to eliminate soil contaminants. It is highly probable that the disease would become more prevalent and severe in fields planted to rice year after year.

Irrigation water, soil, and fragments of plant tissues should not be transferred from a *Fusarium*-infected to a clean area. The disease may also be carried sparingly by the wind and rain, and by man, work animals, tools, and implements or by other mechanical means.

Rice varieties that show considerable resistance should be grown instead of the susceptible sorts. Resistance could be maintained by constant selection.

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# ILLUSTRATIONS

## PLATE 1

- FIG. 1. A rice plant killed by a *Fusarium* disease collected in the field.
2. A close-up of a naturally infected plant showing the fungous growth and sporodochial masses along the margins of the leaf sheaths.
  3. A poured plate culture on potato glucose agar showing the characteristic growth of the fungus colonies.

## PLATE 2

Camera-lucida drawings; all X 880.

- FIG. 1. Microconidia of the Philippine rice *Fusarium*, spindle to ovoid in shape, few in chain, from mycelium of 33-day-old potato-tuber plug culture.
2. Typical slender, sickle-shaped, attenuate, pedicellate macroconidia from pionnotes and mycelium of 33-day-old potato-tuber plug culture and Canadian wonder bean pod culture.
  - 3 and 4. Microconidia and macroconidia germinated in water showing enlargement of the cells of the latter and constriction at the septa. About 2 days old.
  5. Portions of conidiophore, from mycelium of 33-day-old Canadian wonder bean pod culture.

## PLATE 3

- FIG. 1. Rice plants which succumbed to the disease by artificial infection with the Philippine rice *Fusarium*, as compared with normal plants to the right.
2. A close view of an inoculated stool showing a bloom of the fungus at the lower portion.
  3. Two stools of rice showing similar effects of the inoculum.

## PLATE 4

- FIG. 1. Rice plants of the same age at right artificially inoculated with the Philippine rice *Fusarium* to show accelerated growth and partial infection due to the disease as compared with the healthy plants to the left.
2. Two tillers of a rice plant isolated from the rest, to show the wilting effect of the rice *Fusarium*. Note the withered condition of the tiller to the left compared with the unaffected one to the right.
  3. Rice plants inoculated with the same organism exhibiting partial wilting.

## PLATE 5

- FIG. 1. Rice stems with the sheaths partly removed to show the accumulations of the fungus at the nodes due to the rice *Fusarium*.
2. Infected rice stems split into halves to show the internodal and nodal development of the fungus.

## PLATE 6

Type of root observation boxes used in inoculating the roots of rice with the rice *Fusarium*. The three boxes to the right show inoculated plants without any apparent effect.





PLATE 1.



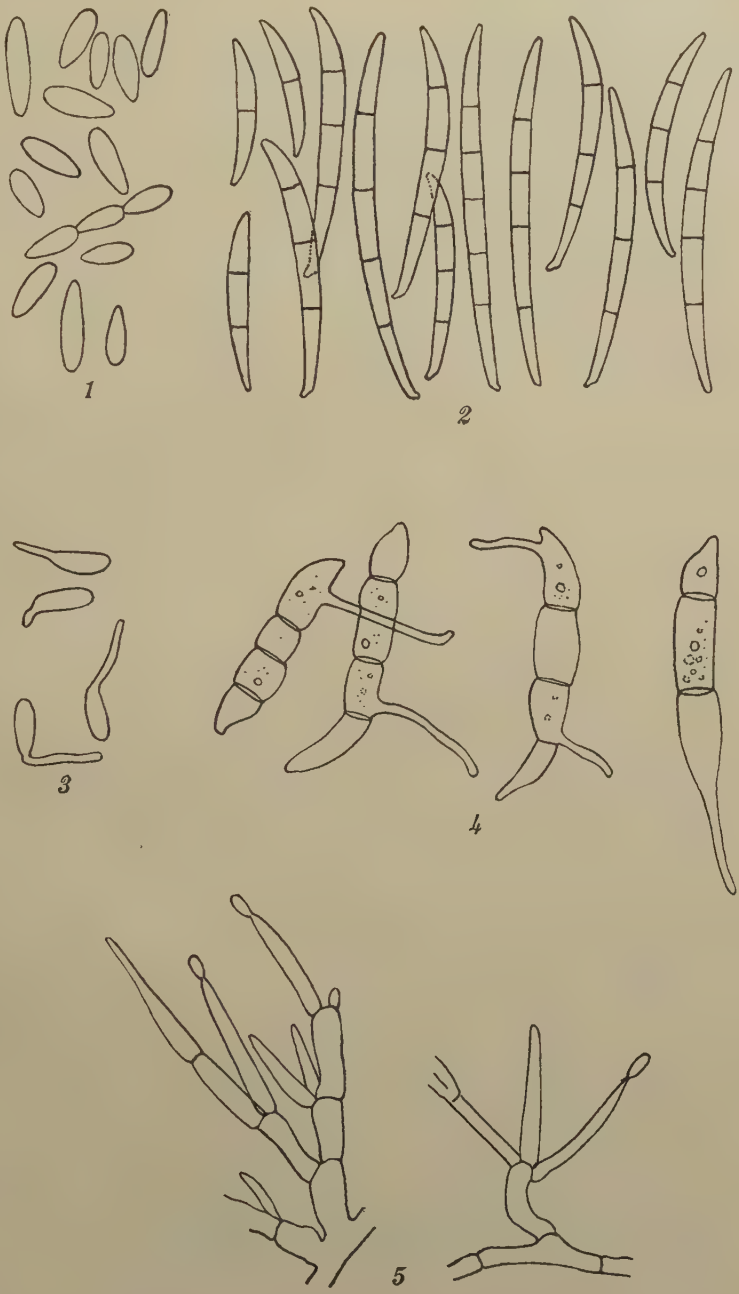


PLATE 2.





PLATE 3.





PLATE 4.



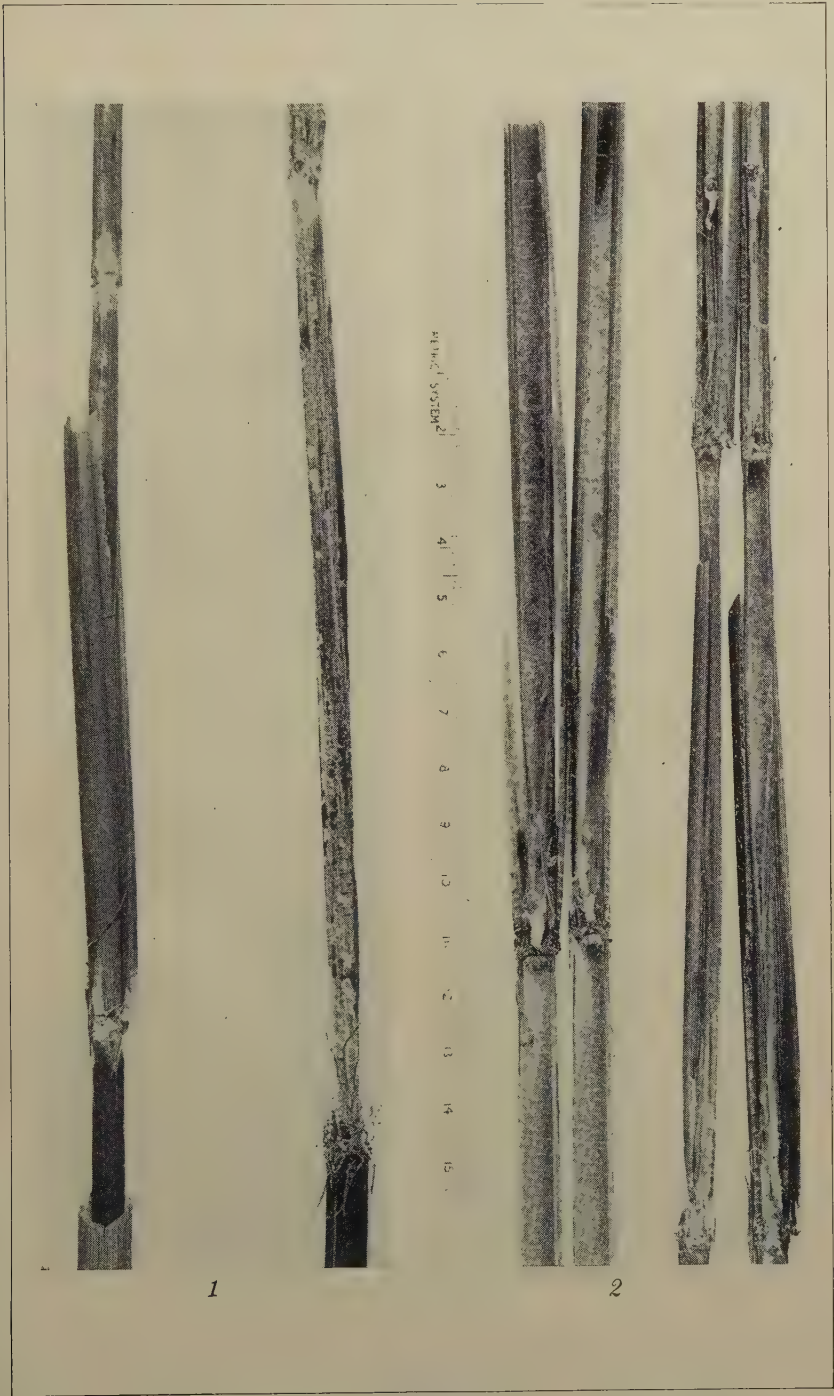


PLATE 5.





PLATE 6.



# A SERIOUS LEAF SPOT OF CHINESE CELERY CABBAGE, WONGBOK, AND OTHER CRUCIFEROUS PLANTS IN TRINIDAD VALLEY, MOUNTAIN PROVINCE, LUZON.<sup>1</sup>

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## FOUR PLATES

### INTRODUCTION

The chinese celery cabbage, or Wongbok, *Brassica pekinensis* Rupr., is an important cruciferous plant commercially grown in Trinidad Valley and in the vicinity of Baguio, Mountain Province, Luzon. Under certain conditions more profit is derived from wongbok than from cabbage, *Brassica oleracea* var. *capitata* Linn., because the former requires less care and fertilizer to produce a good crop than the latter.

In a survey of plant diseases Fajardo(3) reported a gray leaf spot of wongbok and other cruciferous plants, which is common and prevalent in the fields and in the glasshouse seed beds in Trinidad Valley and the environs of Baguio. This disease causes grayish brown lesions identical with the gray leaf spot, caused by *Alternaria herculea* (Ellis and Martin) Elliot, on crucifers in California, which was described by Weimer(9) in 1926.

Whether the disease is indigenous or not, or how long it has existed in the Philippines, nobody knows. In our belief it is of foreign origin and was brought into the Islands some years ago. Since it is serious on the Chinese celery cabbage, or wongbok, and found affecting many other important cruciferous plants, this paper aims to describe it and its causal organism and to present the results thus far obtained.

### HISTORY, GEOGRAPHIC DISTRIBUTION, AND ECONOMIC IMPORTANCE

The gray leaf spot of crucifers was first reported by Ellis and Martin(1) affecting horse-radish, *Armoracia rusticana*

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<sup>1</sup>This investigation was undertaken while the writers were connected with the Bureau of Science, Manila.

Gaertner, in New Jersey in 1882, and the causal organism was given the name *Macrosporium herculeum* (Ellis and Martin). The same fungus was later found by Stewart(6) on flat turnip, *Brassica rapa* Linn., and on cabbage, in Long Island, New York. The author described the spots on the leaf as circular, dead, and brittle. If the spots are numerous the leaf withers and falls off. Schwarze, (5) in his studies on the parasitic fungi of New Jersey, listed *M. herculeum*, causing gray to brown spots on turnips, horse-radish, and other crucifers. In 1926, Weimer(9) reported a leafspot on Chinese cabbage, radish (*Raphanus sativus* Linn.), turnip, and cauliflower (*B. oleracea* var. *botrytis* Linn.), in California, where it is caused by *A. herculea* (Ellis and Martin) Elliot, (*M. herculeum*) producing light gray spots on Chinese cabbage, radish, and turnip, and gray to dark brown spots on cauliflower and cabbage. According to him this disease is known also to occur in Florida, Alabang, Indiana, Ohio, Connecticut, and probably elsewhere.

In the Philippines this disease has a limited distribution, having been found only in Trinidad Valley and in the environs of Baguio, Mountain Province, where the climate is moist and cool. In this region it is serious on wongbok, and less so on pechay, turnip, and other cruciferous plants. Under favorable conditions and especially during the warmer and moister months of the year, 100 per cent infection in the field (Plate 1, fig. 4) or in greenhouse seed beds is not uncommon, and due to the disease from 20 to 50 per cent of the leaves may be badly spotted and culled from the mature plant with a head. In farms where no spraying is practiced and the disease left unchecked, infected plants may dry up and die early, resulting in the failure of the crop. Since the leaves are affected, head formation is naturally decreased in proportion to the amount of foliage attacked.

#### GENERAL SYMPTOMS

All the exposed aërial parts of wongbok and other cruciferous plants at any stage of growth are susceptible to infection. Since only slight differences of symptoms are noted on different crucifers, and since wongbok is most seriously affected, the symptoms on this crop will be described in detail.

On the leaves the disease first appears as small, circular, light brown to brownish violet spots, measuring from less than 0.5 to 1 mm in diameter (Plate 1, figs. 2 and 3). As the disease progresses, greater areas of the leaf blade are involved, the spots becoming either irregular or circular, with grayish to brownish

dead tissue at the center of the lesion (Plate 2, figs. 5 and 6). In this region may be found the spores of the fungus, which, under field conditions, are scantily produced in concentric rings. The lesions often coalesce into larger spots, reaching 10 mm or more in diameter and resulting in the tearing up of the tissues of the blade or death of the leaves (Plate 1, figs. 1 to 3). The lesion on the fleshy midrib usually starts as a small brownish lesion, but as it develops it becomes oblong or linear, sunken, and light brown (Plate 1, fig. 1).

The symptoms on the other crucifers vary slightly in the size and color of the spots on the leaves. On wongbok, pechay, mustard, and radish the lesions are usually light gray to grayish brown and measure from less than 0.5 mm to 10 mm or more in diameter. The spots on mustard, turnip, and radish differ from those on pechay and wongbok only in usually being darker brown and smaller, varying from 0.5 to 5 mm in diameter. On cabbage and broccoli the spots are usually light brown to dark brown and vary from 0.5 to 12 mm in diameter.

#### CAUSAL ORGANISM

The fungus that causes the gray leaf spot of crucifers was first described by Ellis and Martin<sup>(1)</sup> in 1882 on horse-radish (*Nasturtium armoracia* Fries = *Armoracia rusticana* Gaertner), under the name *Macrosporium herculeum* Ellis and Martin.

The authors described the fungus as amphigenous on dark gray round spots; hyphae erect, brown, caespitose, flexuous, sparingly septate, 70 to 80 by 5  $\mu$ ; conidia brown, clavate, multiseptate, with a few imperfect longitudinal septa, 200 to 225 by 21 to 26  $\mu$ . Stewart<sup>(6)</sup> later reported the same fungus on flat turnip and on cabbage in New York, and Schwarze listed it as parasitic on turnips and other crucifers from New Jersey. Elliot,<sup>(2)</sup> in his studies on the taxonomic characters of the genera *Alternaria* and *Macrosporium*, transferred this fungus as it should be to the genus *Alternaria*, and the binomial *Macrosporium herculeum* was changed to *Alternaria herculea* (Ellis and Martin) Elliot. Weimer,<sup>(9)</sup> in 1926, reported gray leaf spot of crucifers in California and identified the causal organism as *Alternaria herculea* (Ellis and Martin) Elliot. He reported the spores as being 125 to 225  $\mu$  long and 16 to 28  $\mu$  wide.

Our measurements of the spores and conidiophores of the fungus obtained from field specimens of diseased fresh leaves of wongbok, pechay, and turnip, showed the fungus in question

to vary in size but is identical with *Alternaria herculea* (Ellis and Martin) Elliot (= *Macrosporium herculeum* Ellis and Martin), described by Ellis and Martin<sup>(1)</sup> and by Weimer.<sup>(9)</sup> On wongbok the conidiophores are 45.6 to 157.7  $\mu$  long, 6.8 to 9.5  $\mu$  wide, and 3 to 7 septate; conidia 144 to 243.2  $\mu$  long, 14.2 to 34.2  $\mu$  wide, and 9 to 15 septate; on pechay conidiophores are 45.6 to 98.8  $\mu$  long, 7.6 to 11.4  $\mu$  wide, and 2 to 4 septate, conidia 121.6 to 247  $\mu$  long, 15.2 to 26.6  $\mu$  wide, and 9 to 16 septate; on turnip conidiophores are 49 to 117.8  $\mu$  long, 7.6 to 11.4  $\mu$  wide, and 3 to 6 septate, conidia 123.7 to 221.8  $\mu$  long, 15.5  $\mu$  to 22.5 wide, and 6 to 16 septate. Plate 3, figs. 1 and 10, and Plate 4, figs. 1, 2, 3, 4, 5, and 12, show conidiophores and conidia of *A. herculea* from various cruciferous hosts. The results are summarized in Table 1.

TABLE 1.—Comparison of the conidiophores and conidia of *Alternaria herculea* from different cruciferous plants.

| Host.                              | Conidiophores. |          |                  | Conidia.    |           |                  |
|------------------------------------|----------------|----------|------------------|-------------|-----------|------------------|
|                                    | Length.        | Width.   | Septa.           | Length.     | Width.    | Septa.           |
|                                    | $\mu$          | $\mu$    |                  | $\mu$       | $\mu$     |                  |
| Horse-radish <sup>a</sup> -----    | 70.0- 80.0     | 5        | ( <sup>c</sup> ) | 200 -225.0  | 21 -26.0  | ( <sup>d</sup> ) |
| Crucifers <sup>b</sup> -----       |                |          | ( <sup>c</sup> ) | 125 -225.0  | 16 -28.0  | ( <sup>d</sup> ) |
| Wongbok <sup>e</sup> -----         | 45.6-157.7     | 6.8- 9.5 | 3-7              | 144 -243.2  | 14.2-34.2 | 9-15             |
| Pechay <sup>e</sup> -----          | 45.6- 98.8     | 7.6-11.4 | 2-4              | 121.6-247.0 | 15.2-26.6 | 9-16             |
| Turnip <sup>e</sup> -----          | 49.0-117.8     | 7.6-11.4 | 3-6              | 123.7-221.8 | 15.5-22.5 | 6-16             |
| Shanghai mustard <sup>e</sup> ---- | 68.4-150.0     | 6.8- 9.5 | 3-7              | 87.4-300.0  | 15.2-28.5 | 7-18             |
| Cabbage <sup>e</sup> -----         | 98.8-171.0     | 5.7- 9.5 | 4-7              | 95.0-264.1  | 11.4-26.6 | 8-11             |
| Broccoli <sup>e</sup> -----        | 45.0- 95.0     | 5.7- 7.6 | 2-5              | 76.0-197.6  | 11.4-22.8 | 7-15             |

<sup>a</sup> Ellis and Martin.

<sup>b</sup> Weimer.

<sup>c</sup> Sparingly.

<sup>d</sup> Multiseptate.

<sup>e</sup> From our measurements of field specimens.

#### COMPARISON OF CONIDIOPHORES AND CONIDIA OF *A. HERCULEA* FROM DIFFERENT CRUCIFERS

*Alternaria herculea* from various infected cruciferous plants in the field or produced from cultures showed wide variation in sizes of conidiophores and conidia. As shown below only *A. herculea* from wongbok and pechay are described in detail.

#### ALTERNARIA HERCULEA FROM WONGBOK

*Conidiophores*.—The conidiophores are produced singly or in groups of two to ten or more on the dead areas on both sur-

faces of the leaves. They are 45.6 to 157.7  $\mu$  long, 6.8 to 9.5  $\mu$  wide, and usually 3 to 7 septate. They are smoke gray or light grayish olive and arise from the hyaline or subhyaline mycelium. They usually have swollen bases and rounded tips, and a few show geniculations. In culture the conidiophores are usually developed from branches or prolongations of the hyaline mycelium which become thickened and light olive gray to deep mouse gray. They are more or less uniform in size and seldom branched.

*Conidia*.—The conidia are borne singly, either apically or on the geniculations of the conidiophores. They are obclavate, straight or slightly curved, beaked, and grayish olive. They measure 144 to 243.2  $\mu$  in length, 14.2 to 34.2  $\mu$  in width, and are usually 9 to 15 septate (Plate 3, figs. 1 to 15; Plate 4, figs. 1 to 3). In culture the conidia are borne either singly or in chains on the tips or on the geniculations of the conidiophores. In the formation of the chain two or more spores may be budded out from the terminal end or from the side of each conidium. They vary from 76 to 216  $\mu$  in length and from 19 to 26.6  $\mu$  in width. They are nonmuriform or submuriform; olive buff, becoming grayish olive and muriform in age.

#### ALTERNARIA HERCULEA FROM PECHAY

*Conidiophores*.—The conidiophores are produced singly or in groups of two or more on both surfaces of the lesion. They are light grayish olive to grayish olive. They are generally short, measuring 45.6 to 98.8  $\mu$  in length and 7.6 to 11.4  $\mu$  in width, and are usually 2 to 4 septate. They may be straight, wavy, and geniculate, with a prominent scar at each geniculation. In culture the conidiophores are developed from thickened and colored mycelium, quite like those found in nature.

*Conidia*.—The conidia are borne singly at the apex or at the geniculation of the conidiophores. They are obclavate, straight or slightly curved, beaked, and muriform. They are 121.6 to 247  $\mu$  long, 15.2 to 26.6  $\mu$  wide, and usually 9 to 16 septate. (Plate 3, figs. 8 to 10; Plate 4, figs. 4 and 5). The spores are light grayish olive to grayish olive. In culture the conidia are borne singly or in chains at the apices or at the geniculations of the conidiophores. They maintain the same shape and form but are slightly larger than those found in nature, being 83.6 to 269.8  $\mu$  long and 15.2 to 30.4  $\mu$  wide and usually 7 to 9 septate.

## ALTERNARIA HERCULEA FROM CABBAGE

*Conidiophores*.—The conidiophores occur singly or in groups of two to five. The young conidiophores are light grayish olive. With age the lower portion usually turns grayish olive, usually straight with basal cells wider than the cells. In some conidiophores the first lower half is wider than the upper cells and seldom geniculate. They are 98.8 to 171  $\mu$  long, 5.7 to 9.5  $\mu$  wide, and usually 4 to 7 septate.

*Conidia*.—The conidia are obclavate, straight or slightly curved, grayish olive, muriform, beaked, 95 to 264.1  $\mu$  long, 11.4 to 26.6  $\mu$  wide, and usually 8 to 18 septate.

## ALTERNARIA HERCULEA FROM BROCCOLI

*Conidiophores*.—The conidiophores are generally short, light grayish olive, occasionally branched, and occur singly or in groups of two to five. A few are geniculate with a scar at each geniculation. They are 45 to 95  $\mu$  long, 5.7 to 7.6  $\mu$  wide, and usually 2 to 5 septate.

*Conidia*.—The conidia are borne apically or at the geniculation on the conidiophores. They are obclavate, straight or slightly curved, beaked, muriform, grayish olive, 76 to 197.6  $\mu$  long, 11.4 to 22.8  $\mu$  wide, and usually 7 to 15 septate.

## HOW TO DISTINGUISH THE GRAY LEAF SPOT FROM OTHER LEAF SPOTS OF CRUCIFERS

This disease can be distinguished from the two other well-known leaf spot diseases of crucifers—ring spot, *Mycosphaerella brassicicola* (Fr.) Lindau and black leaf spot, *Alternaria brassicæ* (Berk.) Sacc.—not only by the difference in the morphology of the causal organism, but also by the type of lesions produced.

The lesions produced by the ringspot disease, *M. brassicicola*, are at first dark purple spots, as they mature they become dark brown with light green borders.<sup>(4)</sup> Later the pycnidia or perithecia of the fungus arranged in concentric rings are produced on the old lesions. The lesions produced by the black leaf spot, *A. brassicæ*, are distinct, roundish, black spots, quite indistinguishable from the ringspot; as the lesions become older, they become grayish to brown, and when spores are abundantly produced, the spots become black to velvety. Spores are produced in circles, giving to the lesions the characteristic concentric rings. The lesions produced by the gray leaf spot *A. herculea*

resemble these two leaf spots in size and shape and in the production of spores in concentric rings. They are, however, much lighter than the ringspot and no pycnidia or perithecia are produced. The gray leaf spot differs from the black leaf spot in having smaller and lighter spots and produces scanty spores under ordinary field conditions. Under humid conditions the spores are also abundantly produced, giving the spots a velvety appearance, so that the disease can be distinguished from the black leaf spot only by means of examination of the spores under the microscope.

The conidia of *A. herculea* are obclavate, straight, or slightly curved, 144 to 243.2  $\mu$  long, 14.2 to 34.2  $\mu$  wide, and 9- to 15-septate, depending upon the host. *Alternaria brassicæ* conidia are usually elongate, fusoid, clavate, 60 to 80  $\mu$  long, 14 to 18  $\mu$  wide, and 6 to 8 muriform-septate.<sup>(7)</sup> According to Weimer<sup>(8,9)</sup> the largest conidia of *A. brassicæ* are 35 to 75  $\mu$  long, 9 to 16.8  $\mu$  wide, and 5 to 8 septate. Fresh diseased specimens of cabbage plants infected with black leaf spot showed the spores to be 19.4 to 87.5  $\mu$  long, 8.7 to 18.1  $\mu$  wide, and 1 to 8 septate. In culture *A. brassicæ* spores are 17 to 57  $\mu$  long, 9 to 17  $\mu$  wide, 1 to 7 septate. The ring spot fungus differs from the above organisms by the production of pycnidia or perithecia. According to Weimer<sup>(8)</sup> the pycnosporos measure 1 to 2.75  $\mu$  long and 3 to 7.5  $\mu$  wide, while the aconosporos measure 3.5 to 5.5  $\mu$  long and 15 to 25  $\mu$  wide.

#### CULTURAL STUDIES

The results as summarized in Table 1 show variations of size and shape of conidia and conidiophores; this variation held true, whether the fungus was obtained from fresh disease specimens or from artificial culture media. Table 2 shows the sizes of conidiophores and conidia of *A. herculea* from wongbok and pechay grown on potato-glucose agar for twenty days. In these cultural studies, differences in the rate of growth and the color of the colony were also noted when the fungus was grown on potato-glucose agar, corn-meal agar, and oatmeal agar. *Alternaria herculea* from wongbok, pechay, and turnip grew more slowly, and the production of spores was delayed and scanty, while *A. herculea* from cabbage and broccoli grew faster and produced more abundant spores. The fungus colonies produced from pechay, wongbok, and turnip are pinkish white, while those on cabbage and broccoli are of a dull or brownish color.

TABLE 2.—Comparison of conidia produced in nature and on potato-glucose agar by *A. herculea* from wongbok and pechay.

| Source of <i>A. herculea</i> . | Conidia.   |           |          |
|--------------------------------|------------|-----------|----------|
|                                | Length.    | Width.    | Septate. |
|                                | $\mu$      | $\mu$     | $\mu$    |
| Wongbok:                       |            |           |          |
| Natural.....                   | 144 -243.2 | 142 -34.2 | 9-15     |
| Artificial.....                | 76 -216.0  | 19 -26.6  | 7-15     |
| Pechay:                        |            |           |          |
| Natural.....                   | 21.6-247.0 | 15.2-26.6 | 9-16     |
| Artificial.....                | 83.6-269.8 | 15.2-30.4 | 7-19     |

Until further studies have been made to prove the existence of strains or forms of the fungus, the gray leaf spot of these cruciferous hosts will be considered as being due to the same morphologic form of *A. herculea* Ellis and Martin. Table 3 shows the rate of growth of each colony on different media at room temperature, 28° to 32° C.

TABLE 3.—The rate of growth of *A. herculea* from different cruciferous hosts on various media (20-day-old colony at room temperature, 28° to 32° C.).

| Source of <i>A. herculea</i> . | Rate of growth per day. |                      |               |
|--------------------------------|-------------------------|----------------------|---------------|
|                                | Corn-meal agar.         | Potato-glucose agar. | Oatmeal agar. |
|                                | cm.                     | cm.                  | cm.           |
| Wongbok.....                   | 1.0                     | 3.5                  | 1.1           |
| Pechay.....                    | 0.5                     | 3.2                  | 1.8           |
| Turnip.....                    | 1.5                     | 1.6                  | 1.2           |
| Cabbage.....                   | 7.5                     | 9.0                  | 9.0           |
| Broccoli.....                  | 6.5                     | 9.0                  | 9.0           |

#### HOST RANGE

Weimer(9) reported cauliflower, cabbage, Chinese cabbage, radish, and turnip affected by this fungus. In his inoculation studies he successfully infected cauliflower, cabbage, and Chinese cabbage with *A. herculea* isolated from Chinese cabbage and rutabaga-infected cabbage. The same fungus was repeatedly isolated from the field and readily infected wongbok, pechay, radish, turnip, mustard, cauliflower, cabbage, and cruciferous plants in Trinidad Valley and the environs of Baguio. Since no cruciferous plants of economic importance are grown in Tri-

nidad Valley other than those mentioned above, no extensive cross inoculations were made to determine other hosts affected by this disease.

#### SEASONAL DEVELOPMENT OF THE DISEASE IN TRINIDAD VALLEY

No experiment has so far been conducted to determine how the fungus tides over from one season to another, but in Baguio the disease is found at all times of the year when cruciferous plants are grown. It is most serious in October, November, December, January, February, May, June, and July, and least serious during March and April, when it is usually hotter and drier. During the hotter and drier "summer" months a successful crop may be obtained without the use of frequent sprays. Because of the favorable temperate climatic conditions, the parasite undoubtedly remains in an active state all the year round in Baguio and vicinity. Its absence in the lowland provinces where other susceptible crucifers are also grown may be due to the differences in climatic conditions.

#### CONTROL MEASURES

Weimer (9) did not consider this disease severe enough in California to justify control measures. In Baguio and Trinidad Valley it is a serious problem that for successful production of wongbok and other cruciferous crops a certain spray must be applied.

A combination spray of Bordeaux mixture, arsenate of lead, and a Japanese insecticide, "Imazu," checks this disease and the injury from flea beetles. Mr. Ishihara, a Japanese farmer in Trinidad, obtained satisfactory results by spraying wongbok plants every three or four days with 2.5-2.5-50 Bordeaux mixture to each 5 gallons of which 80 to 100 grams of lead arsenate and 50 to 80 grams of imazu powder are added. In our preliminary control experiments conducted in 1932, 2-2-50, 2.5-2.5-50, and 3-3-50 Bordeaux mixtures, to each 5 gallons of which 80 grams of imazu and 100 grams of lead arsenate were added, were applied at 4- or 5-day intervals during the growing season; this treatment proved very satisfactory. Trials with 4-4-50 and 5-5-50 concentrations of Bordeaux mixture cause burning of the leaves. Until a better spray has been found, the Bordeaux mixture-lead arsenate-imazu combination spray is recommended.

In view of the short seasonal rotations of cruciferous crops in this region, and since the climate of Baguio is favorable for

continued activity of the parasite, good cultural practices, sanitation, and longer rotation of crops should be practiced. The use of clean seeds and healthy seedlings for field planting to prevent the disease from having an early start in the field, is to be encouraged.

#### SUMMARY

1. A gray leaf spot identical with that described in California by Weimer is reported serious on the Chinese celery cabbage or wongbok and other crucifers in Trinidad Valley and the environs of Baguio, Mountain Province, Luzon.

2. The disease is prevalent, causes 100 per cent infection in the field, and under favorable conditions, from 20 to 50 per cent of the leaves of a single mature plant with a head may be badly affected and drop off.

3. The symptoms are described in detail on wongbok; the lesions are grayish brown to brown and from less than 0.5 mm to 10 mm in diameter. Variations of symptoms were noted on various cruciferous hosts.

4. Measurements of conidiophores and conidia of the fungus from infected leaves of various crucifers in Trinidad Valley showed it to be identical with *A. herculea* Ellis and Martin, which causes gray leaf spot of Chinese cabbage and other crucifers in California.

5. Cultural differences as well as differences in sizes of conidiophores and conidia were noted in *A. herculea* isolated from various hosts. Until further studies have been made, *A. herculea* from wongbok, pechay, and other crucifers will be considered the same morphologic form of *A. herculea* Ellis and Martin.

6. Wongbok, pechay, mustard, turnip, radish, cabbage, and broccoli were found naturally infected by *A. herculea* in Trinidad Valley and the environs of Baguio, Mountain Province.

7. Since infected crucifers can be found at any time of the year the fungus apparently remains active all the year round. The disease is usually more serious during the cooler and moister months of the year than during the summer months.

8. This disease can be checked by spraying with 2-2-50 to 3-3-50 Bordeaux mixture. As the flea beetle is also a serious pest, a combination spray consisting of Bordeaux mixture-lead arsenate-imazu combination spray gave satisfactory results in Baguio. Other measures of control are suggested.

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## ILLUSTRATIONS

### PLATE 1

Photographs showing symptoms of gray leaf spot on Chinese celery cabbage wongbok, *Brassica pekinensis* Rupr.

- FIG. 1. Leaf of a naturally infected wongbok plant showing the typical small light brown to dark brown, young lesions on the blade and on the petiole.
2. Wongbok head showing severe infection of the outer leaves. Some of these leaves dried up due to the disease.
  3. Wongbok leaf from a formed head showing typical tearing and drying up of the leaves due to coalescence of the spots.
  4. View of a wongbok plot in Trinidad Valley, Mountain Province, which showed 100 per cent infection; 10 to 20 per cent of the leaves were badly infected and culled from the head.

### PLATE 2

Photographs of leaves of various cruciferous plants showing typical symptoms of gray leaf spot.

- FIG. 1. Lesions on turnip leaf, *Brassica rapa* Linn.; about  $\times 0.5$ .
2. Lesions on Chinese mustard leaf, *Brassica juncea* Coss.; about  $\times 0.5$ .
  3. Lesions on broccoli leaf, *Brassica oleracea* var. *italica* Plenck.; about  $\times 0.5$ .
  4. Lesions on cabbage leaf, *Brassica oleracea* var. *capitata* Linn.; about  $\times 0.5$ .
  5. Old lesions on wongbok leaf *Brassica pekinensis* Rupr.; about  $\times 1$ .
  6. Lesions on pechay leaf, *Brassica chinensis* Linn.; about  $\times 1$ .

### PLATE 3

Conidia and conidiophores of *Alternaria herculea* grown on potato-glucose agar.

- FIG. 1. A chain of three conidia, from wongbok, *Brassica pekinensis* Rupr.
2. A chain of two conidia with lower conidium bearing side conidiophores and conidia, from wongbok, *Brassica pekinensis* Rupr.
  3. A chain of two conidia borne on the geniculation, from wongbok, *Brassica pekinensis* Rupr.
  4. Conidia borne on the tip and on the geniculation of the conidiophore, from wongbok, *Brassica pekinensis* Rupr.
  5. A mature conidium, from wongbok, *Brassica pekinensis* Rupr.
  6. A young chain of two conidia, from flat turnip *Brassica rapa* Linn.

FIG. 7. Lateral conidiophores borne on a mature conidium, from flat turnip, *Brassica rapa* Linn. Note the conidia borne on the side conidiophores.

8. A young conidium, from pe-tsai, *Brassica chinensis* Linn.

9. A mature conidium, from pe-tsai, *Brassica chinensis* Linn.

10. A chain of two conidia, from pechay, *Brassica chinensis* Linn.

#### PLATE 4

Conidiophores and conidia of *A. herculea* and *A. brassicæ* from various cruciferous plants from the field.

FIG. 1. Conidia of *A. herculea* from wongbok, *Brassica pekinensis* Rupr.

2. Mature conidium of *A. herculea* from wongbok, *Brassica pekinensis* Rupr.

3. Conidiophores of *A. herculea* from wongbok, *Brassica pekinensis* Rupr.

4. Young and mature conidia of *A. herculea* from pechay, *Brassica chinensis* Linn.

5. Groups of conidiophores from pechay, *Brassica chinensis* Linn. Note the geniculations on the conidiophores.

6. Chain of conidia of *A. brassicæ* from cabbage, *Brassica oleracea* var. *capitata* Linn. produced on potato glucose agar.

7. Groups of mature and young conidia of *A. brassicæ* from infected leaves of cabbage in the field.

8. A group of conidiophores of *A. brassicæ* on cabbage showing conidial scars at geniculations. (From lesions.)

9. Branched conidiophore of *A. brassicæ* on cabbage with young conidia borne on the tips.

10. A mature conidium of *A. brassicæ* on cabbage, *B. oleracea* var. *capitata* Linn. with conidiophores borne laterally on the segments. Note the conidia borne on these lateral conidiophores.

11. A conidium of *A. brassicæ* on cabbage showing a prominent beak with conidial scars.

12. Conidia of *A. herculea* obtained from the diseased leaf of turnip, *B. rapa* Linn.

(All drawings were made with the aid of a camera lucida. Magnified about 400 times.)



1



2



3



4

PLATE 1.





PLATE 2.



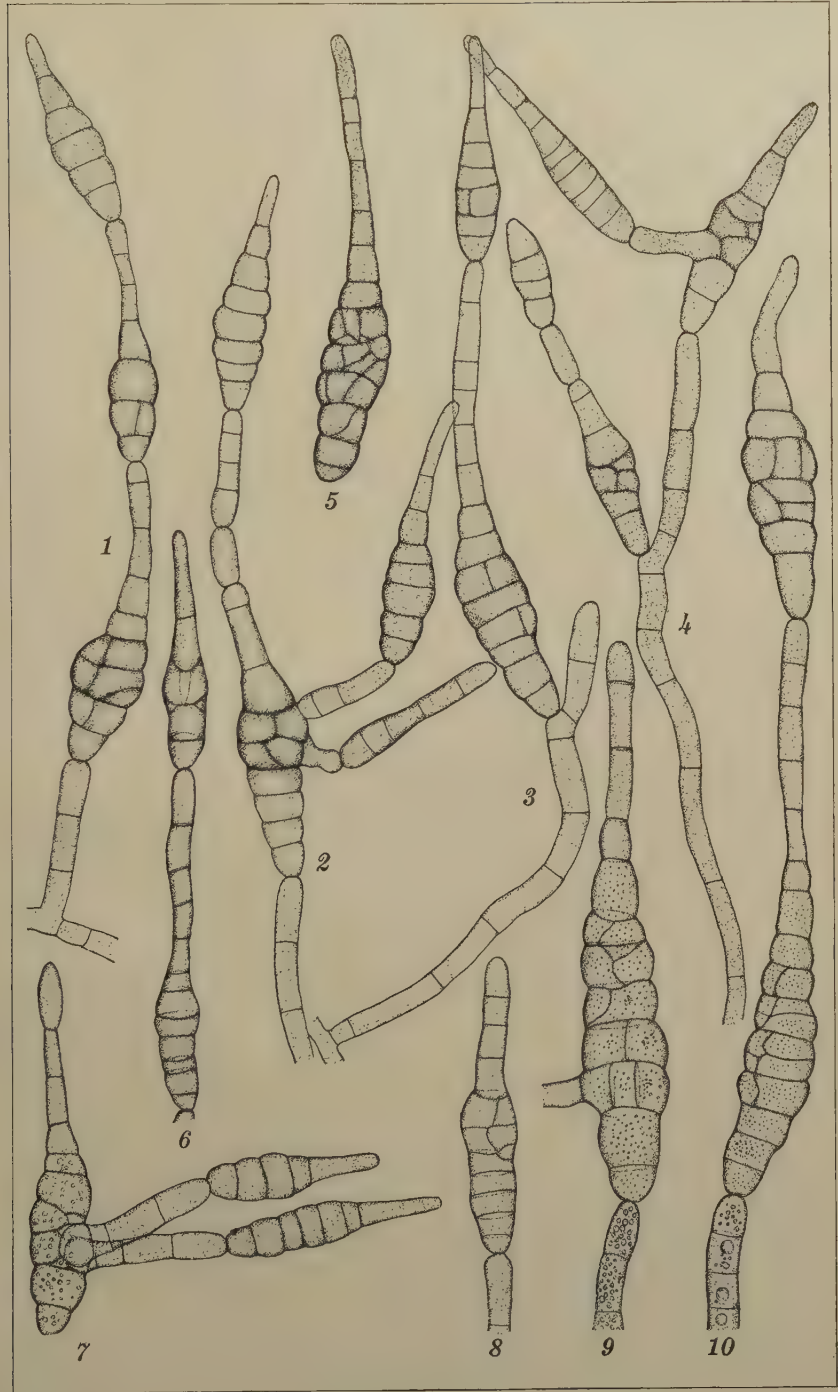


PLATE 3.



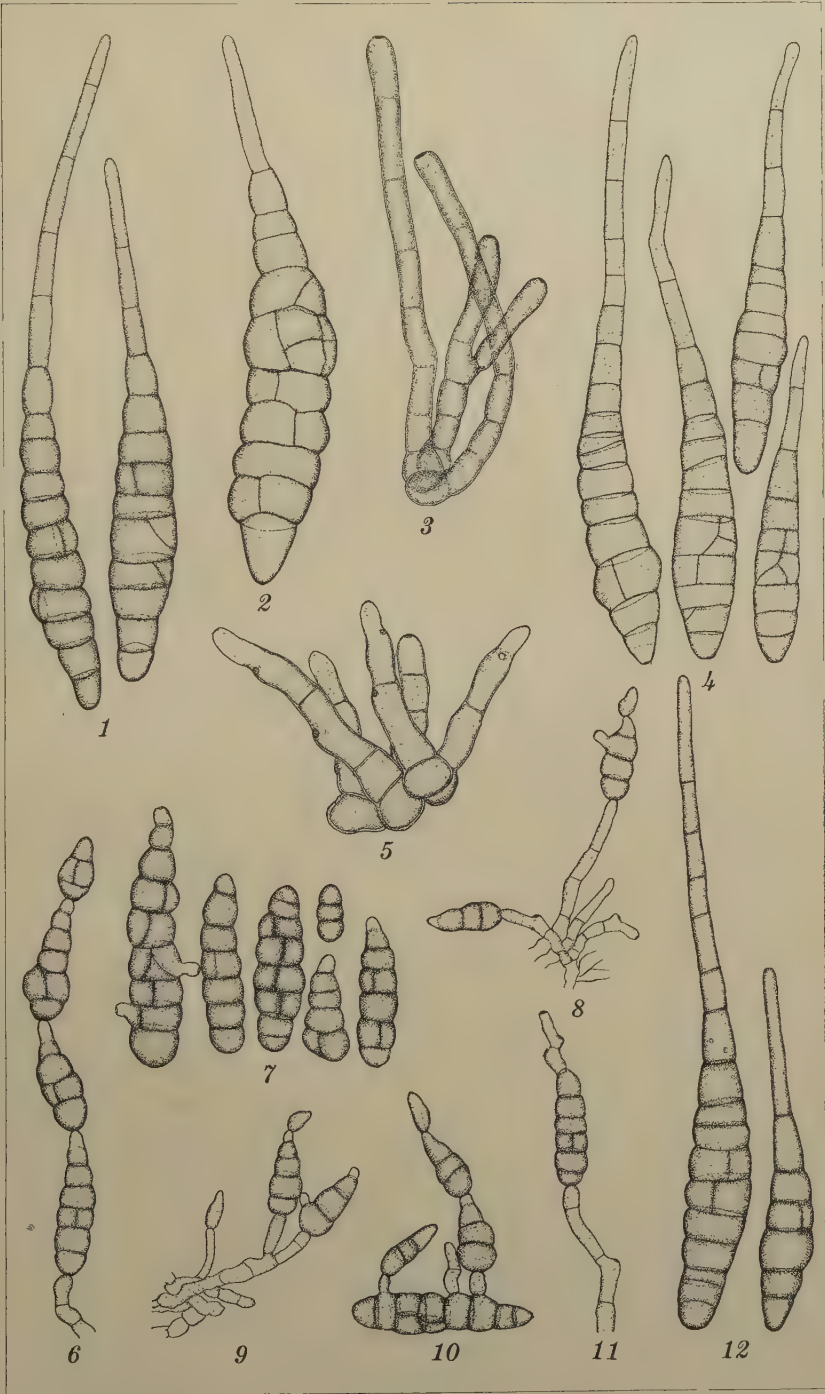


PLATE 4.



# THE RELATIVE EFFICACY OF DIFFERENT STORAGE SOLUTIONS FOR THE PRESERVATION OF NATURAL COLOR IN PHILIPPINE PLANT MATERIAL FOR EXHIBITION PURPOSES.<sup>1</sup>

By F. T. ADRIANO and D. P. TABIJE

## ONE PLATE

In a former paper, published in the Natural and Applied Science Bulletin, we described tentative solutions for preserving the natural colors of plant materials. Subsequent experiments with a large variety of fruits and vegetables, and an investigation covering a period of more than a year, have given results which necessitate the presentation of another paper to explain the preparation of more satisfactory solutions for preserving natural colors of plant materials.

Preserving solutions recommended for temperate materials did not prove satisfactory for the preservation of natural color in Philippine fruits and vegetables.

However, by using the formulæ recommended by other investigators in foreign countries and modifying them whenever necessary to suit Philippine materials, we were able to secure preserving solutions that may be considered satisfactory.

## A. MATERIALS

### I. REAGENTS AND SOLUTIONS

1. *Saturated copper sulphate solutions prepared by dissolving 400 grams in 1 liter of water.*—This serves as a stock solution that can be used over and over again. After long use its strength will greatly diminish, but if more copper sulphate is added, it can be used like the fresh stock.

2. *Acetate of copper dissolved to saturation in strong acetic acid solution (one part of the solution being diluted to four parts of water).*—The specimens are immersed for fifteen minutes in this solution heated to the boiling point and then washed in clean water for a few minutes until free from all traces of surface

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<sup>1</sup> Submitted for publication January, 1934.

deposits of acetate of copper (Trail's fixing solution). They are transferred later to any preservative liquid desired.

3. *Sulphurous acid solution, 6 per cent strength.*—This is prepared by passing sulphur dioxide gas from a storage cylinder into distilled water to the saturation point.

4. Formalin solution, 40 per cent strength.
5. Boric acid powder.
6. Refined salt. (In the absence of refined salt, ordinary clean table salt may be used.)
7. *Potassium nitrate crystals.*
8. Glycerine, or 15 per cent sugar solution.
9. Alcohol, 95 per cent.
10. Zinc Chloride crystals.
11. Brine solution, 10 per cent.
12. Distilled water. (In the absence of distilled water, filtered faucet or tap water or rain water may be used.)
13. Museum jars or containers.
  - a. Special museum jars.
  - b. Other kinds of glass jars provided with tight-fitting covers.

14. *Plant materials, different kinds of fruits and vegetables.*—For natural-color preservation, it is very important to use freshly gathered specimens of fruits and vegetables that are perfect in shape, color, and condition. Perfect specimens, of large size and free from blemishes, should be selected.

#### B. METHODS OF NATURAL COLOR PRESERVATION

No single solution or even a combination of solutions, has been found satisfactory for all kinds of colored or uncolored materials. For convenience, the specimens for natural-color preservation should be classified into groups, such as (a) green-colored, and (b) multicolored samples.

1. *Green-colored specimens.*—In the preservation of green-colored specimens, two solutions are needed: (a) a fixing solution and (b) a storage solution.

(a) *Fixing solution.*—The specimens are cleaned in water and immersed either in the copper sulphate solution or in Trail's fixing solution to fix the green color (chlorophyll) of the plant tissues. Overimmersion is usually indicated by the formation of brownish or burnt-looking spots. Immersion usually takes from two to three days. In some cases, a few hours' immersion will suffice.

After immersion in the fixing solution, the materials are washed very thoroughly in clean water for several hours or longer, until all excess copper sulphate or acetate of copper is

removed. The completely washed specimens are then stored in glass or museum jars and filled immediately with the storage solution.

(b) *Storage solution*.—There are fourteen kinds of storage solutions. These are designated as formula 1, 2, 3, and so on. The museum jar or bottle should be filled with storage solution to 1 or 1.5 inches below the lip, and the specimens should be completely submerged in the solution, after which the container is carefully sealed to exclude air and dust, and to prevent the loss of sulphur dioxide, etc. If the storage solution becomes cloudy, it should be changed with a new one.

2. *Multicolored specimens or specimens of any color including green*.—In the preservation of this kind of specimens both fixing and storage solutions are needed. Two fixing formulæ are used that are observed to be good for all specimens of any color, including green. Both are good, and they are prepared as follows:

*Fixing formula A.*

|                                                          |         |
|----------------------------------------------------------|---------|
| 1. Distilled water, cc                                   | 1,000.0 |
| 2. Formalin, 40 per cent, cc                             | 1.0     |
| 3. Boric acid powder, g                                  | 7.5     |
| 4. Copper sulphate solution to give a faint green color. |         |

*Fixing formula B.*

|                                                          |         |
|----------------------------------------------------------|---------|
| 1. Distilled water, cc                                   | 1,000.0 |
| 2. Boric acid powder, g                                  | 7.0     |
| 3. Potassium nitrate, g                                  | 1.0     |
| 4. Sugar solution, 15 per cent, cc                       | 42.5    |
| 5. Copper sulphate solution to give a faint green color. |         |

Specimens of any color, the preservation of which is desired, should be cleaned thoroughly in water and immersed for one or two weeks in any one of these fixing solutions in well-closed museum jars, or until slight browning of the specimens appears. They are then to be removed from the fixing solution and again washed in clean water. Immediately after washing they should be packed and immersed in a storage solution of sulphurous acid of 0.1 to 2 per cent strength, depending on the kind of sample. If the sample, after being removed from the fixing solution, shows much browning, a stronger concentration of sulphurous acid solution should be used.

This method is recommended for fruits like green and ripe mangoes, guavas, kalamondin, green oranges, pineapples, persimons, mabolo, hevi, ates, avocados, katmon, lanzones, peaches, karanda, white grapes, chayotes, patola, achuete, etc.

3. *The use of a fungicide.*—To prevent the growth of molds and fungus in the storage solution, fungicides like copper sulphate, phenol, and sulphurous acid (when this is not used for storage solution) are used.

(a) *Sulphurous acid, 6 per cent strength, a trace, may be added to the storage solution in the glass jars, or may be included in the formula while it is being prepared.*—Sulphurous acid, 6 per cent strength, is prepared by passing sulphur dioxide gas into distilled water long enough to give 6 per cent. The strength of sulphurous acid solution is tested with a standard iodine solution.

(b) *Copper sulphate solution is prepared by dissolving copper sulphate crystals in distilled water.*—It is added to the storage of preserving solution to give a faint green color only.

(c) *Phenol or carbolic acid.*—One part of saturated carbolic acid solution to one part of alcohol is added to the preserving solution in the jar in amounts of from 5 to 10 cc. Weaker concentrations were found to be unsatisfactory.

#### C. PRESERVING OR STORAGE SOLUTIONS

The following storage or preserving solutions were tried:

##### Formula 1.

|                                                        |         |
|--------------------------------------------------------|---------|
| 1. Distilled water, cc                                 | 1,000.0 |
| 2. Formalin, 40 per cent, cc                           | 1.0     |
| 3. Sulphurous acid, 6 per cent strength, cc            | 4.0     |
| 4. Boric acid powder, g                                | 7.5     |
| 5. Copper sulphate solution to give faint green color. |         |

##### Formula 2.<sup>a</sup>

|                                                 |          |
|-------------------------------------------------|----------|
| 1. Distilled water, cc                          | 1,000.00 |
| 2. Refined salt, g                              | 14.80    |
| 3. Formalin, 40 per cent, cc                    | 2.00     |
| 4. Sulphurous acid, 6 per cent, cc              | 1.00     |
| 5. Potassium nitrate, g                         | 1.85     |
| 6. Glycerine or sugar solution, 15 per cent, cc | 71.40    |

<sup>a</sup> Cruess recommends this preservative fluid for multicolored specimens.

##### Formula 3.

|                                    |       |
|------------------------------------|-------|
| 1. Sulphurous acid, 6 per cent, cc | 1,000 |
| 2. Boric acid powder, g            | 2-4   |
| 3. Formalin, 40 per cent, cc       | 5-20  |

##### Formula 4.

|                              |       |
|------------------------------|-------|
| 1. Distilled water, cc       | 1,000 |
| 2. Alcohol, 95 per cent, cc  | 150   |
| 3. Formalin, 40 per cent, cc | 20    |

The mixture should be allowed to stand, and if there is any sediment, the clear liquid may be poured off and the remainder filtered through filter paper.

*Formula 5.*

|                             |       |
|-----------------------------|-------|
| 1. Distilled water, cc      | 1,000 |
| 2. Alcohol, 95 per cent, cc | 138   |
| 3. Boric acid, powder       | 24    |

If the solution is not clear it should be allowed to stand and settle; then the clear supernatant portion may be poured off and the remainder filtered through filter paper.

*Formula 6.*

|                               |       |
|-------------------------------|-------|
| 1. Distilled water, cc        | 1,000 |
| 2. Alcohol, 95 per cent, cc   | 134   |
| 3. Zinc chloride, crystals, g | 84    |

The zinc chloride is added to the water and stirred until all is dissolved. Then the alcohol is added. The solution is allowed to stand until clear; then the clear liquid is poured off and the remainder filtered through filter paper.

*Formula 7.*

|                                |   |
|--------------------------------|---|
| 1. Distilled water, parts      | 8 |
| 2. Sulphurous acid, 6 per cent | 1 |
| 3. Alcohol, 95 per cent        | 1 |

The mixture is allowed to stand, and if there is any sediment, the clear liquid is poured off and the remainder filtered. Denatured alcohol is not suitable for this solution.

*Formula 8.*

This preserving solution consists of sulphurous acid ranging from 0.1 to 2 per cent strength. Strong stock solution of sulphurous acid 6 per cent strength can be diluted with water to any desired weaker concentration.

*Formula 9.*

|                                 |       |
|---------------------------------|-------|
| 1. Water, cc                    | 1,000 |
| 2. Copper sulphate, crystals, g | 400   |

The mixture is heated to hasten solution, allowed to settle for a day, and the clear liquid poured off for use.

*Formula 10.*

|                                                 |       |
|-------------------------------------------------|-------|
| 1. Distilled water, cc                          | 1,000 |
| 2. Sulphurous acid, 6 per cent, cc              | 1     |
| 3. Formalin, 40 per cent, cc                    | 2     |
| 4. Refined salt, g                              | 15    |
| 5. Potassium nitrate, g                         | 2     |
| 6. Glycerine or sugar solution, 15 per cent, cc | 60    |

*Formula 11.*

|                                                          |         |
|----------------------------------------------------------|---------|
| 1. Distilled water, cc                                   | 1,000.0 |
| 2. Sulphurous acid, 6 per cent, cc                       | 7.5     |
| 3. Refined salt, g                                       | 22.5    |
| 4. Potassium nitrate, g                                  | 7.5     |
| 5. Copper sulphate solution to give a faint green color. |         |

*Formula 12.*

|                                                    |         |
|----------------------------------------------------|---------|
| 1. Distilled water, cc                             | 1,000.0 |
| 2. Sulphurous acid, 6 per cent, cc                 | 3.8     |
| 3. Boric acid powder, g                            | 7.5     |
| 4. Copper sulphate to give a faint green solution. |         |

*Formula 13.*

|                                                        |         |
|--------------------------------------------------------|---------|
| 1. Distilled water, cc                                 | 1,000.0 |
| 2. Boric acid powder, g                                | 7.5     |
| 3. Potassium nitrate, g                                | 1.0     |
| 4. Glycerine or sugar solution, 15 per cent, cc        | 45.0    |
| 5. Copper sulphate solution to give faint green color. |         |

*Formula 14.*

|                                    |         |
|------------------------------------|---------|
| 1. Distilled water, cc             | 1,000.0 |
| 2. Sulphurous acid, 6 per cent, cc | 7.5     |
| 3. Sugar solution, 15 per cent, cc | 75.0    |

*Formula 15.*

|                                    |          |
|------------------------------------|----------|
| 1. Distilled water, cc             | 1,000.00 |
| 2. Boric acid, powder, g           | 11.24    |
| 3. Formalin, 40 per cent, cc       | 22.50    |
| 4. Refined salt, g                 | 22.50    |
| 5. Sugar solution, 15 per cent, cc | 45.000   |

## DISCUSSION OF RESULTS

The data in Table 1, which were obtained from a series of trials, show the relative efficacy of the different preserving solutions for natural-color preservation of Philippine and some foreign-grown plant materials for museum or exhibition purposes. A large number of semitemperate fruits which came from southern China and the United States, as well as fruits and vegetables grown in the Philippines, were used for this study.

Failures as well as satisfactory results were noted. Column 4 of Table 1 shows that the natural colors of many plant specimens can be satisfactorily preserved in more than one kind of preserving solutions.

Poor results were apparently observed in apples (red and green), hevi, bael, beets, bignay, cabbage (purple), litchi, red and white radishes, serali, and tersana, when solutions recommended by former investigators were used.

TABLE 1.—Efficacy of natural color preserving solution.

| Sample.                                   | Origin.                    | Original color.       | Solutions that gave satisfactory results arranged in the order of the best preservation. | Solutions that gave unsatisfactory results. |
|-------------------------------------------|----------------------------|-----------------------|------------------------------------------------------------------------------------------|---------------------------------------------|
| Alaga; <i>Uvaria sorooponensis</i> Pal.   | Pasay, Rizal               | Green and red.        | 14, 8.                                                                                   | 3, 10, 5.                                   |
| Ampalaya; <i>Momordica charantia</i> L.   | Divisoria Market, Manila   | Green.                | 1, 2, 6, 7, 3                                                                            |                                             |
| Annatto; <i>Bixa orellana</i> L.          | Pasay, Rizal               | Green and dark brown. | 8, 1, 15.                                                                                | 13.                                         |
| Apple; <i>Pyrus malus</i> L.              | Shanghai, China.           | Pale green.           | 12, 13, 8.                                                                               | 1, 2, 3, 5, 7, 10, 14, 15.                  |
| Do.                                       | do.                        | Red and green.        |                                                                                          | 1, 13.                                      |
| Atos; <i>Annona squamosa</i> L.           | Pasay, Rizal               | Pale green.           | 8.                                                                                       | 1, 4, 7, 13.                                |
| Avocado; <i>Persea americana</i> Mill.    | Lamao, Bataan.             | Green.                | 8, 3, 4, 1, 7.                                                                           | 6.                                          |
| Bael; <i>Aegle marmelos</i> Corr.         | do.                        | Pale green.           |                                                                                          | 1, 15.                                      |
| Beet; <i>Beta vulgaris</i> L.             | Shanghai, China.           | Purple.               |                                                                                          | 2.                                          |
| Bignay; <i>Ankidesma buntius</i> Spr.     | Pasay, Rizal               | Red, black, white.    | 15, 4, 5, 2, 10.                                                                         | 14, 1, 3, 6, 7, 8, 11, 12 and 13.           |
| Cabbage; <i>Brassica oleracea</i> L.      | Baguio, Mountain Province. | Purple.               |                                                                                          | 2.                                          |
| Cantaloupe; <i>Cucumis melo</i> L.        | Shanghai, China.           | Light yellow.         | 8.                                                                                       |                                             |
| Carrot; <i>Caucus carota</i> L.           | Hongkong, China.           | Orange.               | 7, 14, 8.                                                                                | 10, 1.                                      |
| Castafias; <i>Castanea mollissima</i> Bl. | Shanghai, China.           | Dark brown.           | 15, 8.                                                                                   |                                             |
| Chayote; <i>Sechium edule</i> Sw.         | Divisoria Market, Manila.  | Light green.          | 13, 1.                                                                                   | 8, 11.                                      |
| Corn, ear; <i>Zea mays</i> L.             | San Andres Market, Manila. | White, yellow.        | 8, 12, 1.                                                                                | 11.                                         |
| Cucumber; <i>Cucumis sativus</i> L.       | do.                        | Green, white crystal. | 1, 2, 2-4 per cent formalin.                                                             | 8, 11, 13.                                  |
| Gabi; <i>Colocasia esculentum</i> L.      | Canton, China.             | Dark brown.           | 8, 11.                                                                                   |                                             |
| Ginger; <i>Zingiber officinale</i> R.     | Divisoria Market, Manila.  | Brown.                | 8, 15.                                                                                   |                                             |
| Do.                                       | Canton, China.             | Dull white.           | 8, 7.                                                                                    |                                             |
| Grape; <i>Vitis rotundifolia</i> Mx.      | California.                | Pale green.           | 8, 7.                                                                                    | 3, 5, 6, 13, 14.                            |
| Do.                                       | do.                        | Purple.               | 2.                                                                                       | 15.                                         |
| Guama; <i>Inga laurina</i> L.             | Lamao, Bataan.             | Green, yellow.        | 8.                                                                                       | 13.                                         |
| Guava; <i>Psidium guajava</i> L.          | Pasay, Rizal               | Green, yellow.        | 13, 8, 1, 11.                                                                            | 10, 2, 7, 5, 6.                             |
| Hevi; <i>Spondias cytherea</i> Son.       | Lamao, Bataan.             | Green.                | 13, 8.                                                                                   | 7, 3, 5, 6.                                 |
| Kalamondin; <i>Citrus mitis</i> Blo.      | Divisoria Market, Manila.  | do.                   | 12, 13, 8, 3, 7.                                                                         | 4, 6, 10, 14, 15.                           |
| Karanda; <i>Carissa carandas</i> L.       | Sampaloc, Manila.          | Black, green.         | 13, 15, 8.                                                                               | 1, 2, 4, 5, 7, 10.                          |

TABLE 1.—Efficacy of natural color preserving solution—Continued.

| Sample.                                     | Origin.                         | Original color.        | Solutions that gave satisfactory results arranged in the order of the best preservation. | Solutions that gave unsatisfactory results. |
|---------------------------------------------|---------------------------------|------------------------|------------------------------------------------------------------------------------------|---------------------------------------------|
| Katmon; <i>Dillenia philippinensis</i> Rfe. | Singalong Experimental Station. | Pale green.            | 8, 14.                                                                                   |                                             |
| Lanzones; <i>Lansium domesticum</i> J.      | Paete, Laguna.                  | Light yellow.          | 8, 7, 1.                                                                                 | 2, 3, 4, 5, 6, 10, 11, 13, 14, 15.          |
| Litchi; <i>Litchi chinensis</i> Jon         | Shanghai, China.                | Crimson, red.          |                                                                                          | 8, 10, 11.                                  |
| Lime; <i>Citrus aurantiifolia</i> C         | Tanauan, Batangas.              | Green.                 | 1, 3, 8, 13, 11, 7.                                                                      |                                             |
| Limonetto; <i>Triphasia trifolia</i> P. W.  | Pasay, Rizal.                   | Green, red.            | 13, 8.                                                                                   |                                             |
| Limon real; <i>Citrus exelsa</i> Wes.       | Divisoria Market, Manila.       | Light yellow.          | 1, 8.                                                                                    |                                             |
| Mabolo; <i>Diospyros discolor</i> Wild.     | Lamiao, Bataan.                 | Red.                   | 8, 10.                                                                                   |                                             |
| Mango; <i>Mangifera indica</i> L.           | Cebu.                           | Green.                 | 1, 8, 13, 2.                                                                             |                                             |
| Do.                                         | Divisoria Market, Manila.       | Yellow.                | 1, 2, 8, 13.                                                                             |                                             |
| Mangosteen; <i>Garcinia mangostana</i> L.   | Shanghai, China.                | Purple.                | 15, 4, 8.                                                                                | 1, 2.                                       |
| Nama.                                       | do.                             | Yellow.                | 15, 8, 13, 1.                                                                            |                                             |
| Okra; <i>Abelmoschus esculentus</i> Mch.    | Divisoria Market, Manila.       | Green.                 | 1, 8.                                                                                    |                                             |
| Orange; <i>Citrus sinensis</i> Osb.         | Tanauan, Batangas.              | do.                    | 1, 3, 8, 13, 11, 7.                                                                      |                                             |
| Do.                                         | Bukidnon, Mindanao.             | do.                    | 1, 13.                                                                                   |                                             |
| Do.                                         | Portland, Oregon.               | Yellow.                | 1, 13, 14.                                                                               |                                             |
| Patola; <i>Luffa cylindrica</i> Roem.       | Divisoria Market, Manila.       | Green.                 | 8, 7.                                                                                    | 1.                                          |
| Peach; <i>Prunus persica</i> S. and Z.      | Shanghai, China.                | Multicolored.          | 1, 2, 8.                                                                                 | 7, 14.                                      |
| Peanut; <i>Arachis hypogaea</i> L.          | do.                             | Yellow.                | 8.                                                                                       |                                             |
| Pear.                                       | Canton, China.                  | Orange.                | 3, 7, 8.                                                                                 | 1, 2, 15, 13.                               |
| Pepper, long; <i>Capiscum annuum</i> L.     | Divisoria Market, Manila.       | Green.                 | 1, 2, 3, 5.                                                                              | 6, 7, 8.                                    |
| Do.                                         | do.                             | Red.                   | 2, 11, 1, 5.                                                                             | 12, 3, 4, 8, 10, 13.                        |
| Persimmon; <i>Diospyros virginiana</i> L.   | Shanghai, China.                | Pale green and orange. | 10, 1, 2, 13, 8.                                                                         |                                             |
| Pineapple; <i>Ananas comosus</i> M.         | Bukidnon, Mindanao.             | Greenish purple.       | 13, 18.                                                                                  |                                             |
| Do.                                         | Cavinti, Laguna.                | Yellow.                | 7, 8.                                                                                    |                                             |
| Plum.                                       | Santao, China.                  | Purplish.              | 2, 1, 15, 8.                                                                             | 4.                                          |
| Potato; <i>Solanum tuberosum</i> L.         | Shanghai, China.                | Brown.                 | 15, 8.                                                                                   |                                             |

|                                             |                                       |                     |                             |
|---------------------------------------------|---------------------------------------|---------------------|-----------------------------|
| Radish; <i>Raphanus sativus</i> L.          | Baguio, Mountain Province.            | Red.                | 1, 2, 4.                    |
| Do.                                         | do.                                   | White.              | 1, 11.                      |
| Rice; <i>Oryza sativa</i> L.                | Singalong Experiment Station, Manila. | Greenish white.     | 1, 5, 7, formalin, alcohol. |
| Santol; <i>Sandoricum koetjape</i> M.       | Novaliches, Rizal.                    | Yellow.             | 1, 13, 15.                  |
| Serali; <i>Flacourtia romontchi</i> V'Her.  | Lamiao, Bataan.                       | Dark red.           | 2, 3, 10.                   |
| Stringbean; <i>Vigna sinensis</i> Fruw.     | Divisoria Market, Manila.             | Pale white.         | 5, 7.                       |
| Do.                                         | do.                                   | Purple.             | 5, 8, 2.                    |
| Sweet potato; <i>Ipomoea batatas</i> L.     | Santao, China.                        | White.              |                             |
| Tersana; <i>Eugenia malaccensis</i> L.      | Lamiao, Bataan.                       | White, red.         | 3, 8.                       |
| Tomato; <i>Lycopersicon esculentum</i> Mil. | San Andres Market, Manila.            | Red, yellow.        | 11, 8, 14.                  |
| Vilatti; <i>Feronia limonia</i> Swg.        | Lamiao, Bataan.                       | White (rough skin). | 14, 5, 1, 4.<br>8, 11.      |

In the course of the experiments it was found that the natural color of some plant materials is difficult to preserve. This difficulty is more apparent in the case of hevi fruits. Greening with copper sulphate solution for longer than thirty minutes bleaches the fruit, especially if the solution is saturated. Greening this fruit with the same solution but using a very weak concentration, requires a longer time of immersion. Satisfactory results were obtained by means of fixing formula B and storing the fruit later in sulphurous acid solution of 0.2 per cent strength.

Another fruit which was found difficult to work with was ates. This fruit may be greened with copper sulphate solution or any of the fixing solutions A and B. Weak sulphurous acid of 0.2 to 0.5 per cent strength is the only solution that was found good for this fruit.

White cucumbers also could not be satisfactorily preserved. Formula 11 recommended by Cruess for preserving white vegetables was tried with white corn, white cucumbers, white radishes, etc., with no success. Fungus grew luxuriantly a week after the operation. However, by using 2 to 4 per cent formalin, a better and more encouraging result was obtained, and the growth of fungus and the water-soaked appearance, associated with some solutions tried, were greatly lessened.

The natural-color preservation of lanzones requires a separate treatment. Soaking the fruit in water from three to six hours after washing and using only weak concentrations of sulphurous acid solution (0.2 to 0.5 per cent), was found to give satisfactory results. Cracking, softening, loss of turgidity, and falling off of the fruits from the fruit stalk, were invariably observed in fruits not subjected to the above treatments. Moreover, the fruits did not fall off when preserving formulas 5, 11, and 13 were used, but in these solutions the natural-color preservation was not satisfactory.

The growth of molds and fungi was noted in all solutions except in formulas 3, 7, and 8 which in most cases affected the decolorization of the specimens as well as the preserving solutions themselves, making an immediate changing of the solution necessary to prevent specimens from becoming discolored or mushy. This has been a common trouble in natural-color preservation, so the need for proper fungicides is apparent.

Three kinds of fungicides, copper sulphate solution, phenol, and sulphurous acid 6 per cent, all added in traces, were used. It was found that copper sulphate solution cannot effectively

check fungous growth. Phenol does it, but its use brings about discolorization, especially when citrus fruits are used. In many instances, traces of sulphurous acid solution, 6 per cent strength, gave better results.

Trail's fixing solution is no better than copper sulphate solution to fix the green color of the specimens, while the fixing formulæ A and B, in all cases prove better than the copper sulphate solution. While some plant pathologists prefer Trail's fixing solution, steeping the specimens in the boiling copper acetate solution requires a degree of skill, vigilance, and technic of which an ordinary man is not usually capable. There is apt to be oversteeping which will result in partial or total bleaching. This is quite a delicate process. Copper sulphate solution is good, but it only fixes green, hence the fixing formulæ A and B, which fix more than one color, are recommended for use.

Of all preserving solutions tried, formula 8 is the best, for obvious reasons. First, it prevents fungous growth, which has been a troublesome feature in the work, and hence requires changing of the solution from time to time. Second, longer time preservation and a better appearance of the specimens were obtained with this than with any other formula tried. Third, it has the widest range of suitability for natural-color preservation, provided the specimens are first subjected to proper color fixation. The concentration to be used varies from 0.1 to 2 per cent, depending upon the texture and quality of the specimens.

Judging from the satisfactory results obtained from the use of different preserving formulæ, a general classification may be made to take care of the five general kinds of colors of plant materials.

A considerable number of trials with formula 8 have already been made and very encouraging results have invariably been obtained thus far. The classification is as follows:

| Color of specimen | Preserving formulæ <sup>a</sup> |
|-------------------|---------------------------------|
| Green             | 8, 1, 13, 2, 3, 7               |
| Black or brown    | 15, 8                           |
| Red or orange     | 8, 10, 14, 7                    |
| Yellow or white   | 8, 13, 1, 2, 3, 7               |
| Multicolored      | 2, 8                            |

<sup>a</sup> The formulæ are arranged in the order of the best preservation obtained in our trials.

#### SUMMARY

1. Two hundred seventy-nine trials were made with fifty-seven kinds of fruits and vegetables representing many colors.

2. It is important that specimens preserved for natural color should be of firm texture. Only ripe but still hard fruits free from injuries and blemishes, and those perfect in shape and color, should be preserved.

3. Of the three methods of fixing the colors of plant materials, fixing formulæ A and B were found to be more satisfactory than Trail's solution.

4. Except in the case of white cucumbers, where only 2 to 4 per cent formalin gives satisfactory results in color preservation, the use of formula 8 was found satisfactory.

5. The use of fungicides in preserving solutions which consist mainly of sulphurous acid or formalin, is unnecessary. In preserving solutions that do not contain a large portion of sulphurous acid or formalin, little or no success was observed when phenol or copper sulphate were used as fungicides.

6. Some fruits are of such texture and delicacy that natural color preservation is very difficult. This is particularly noticed in the case of fruits like the hevi, ates, bignay, litchi, serali, tersana, etc. The best that can be done with them is to use very weak fixing solutions requiring long immersion and frequent changes of storage solution.

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## ILLUSTRATIONS

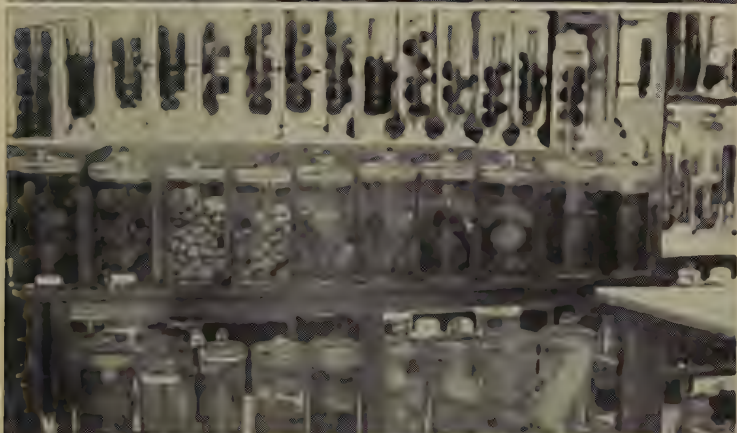
### PLATE 1

- FIG. 1. A corner in the agricultural chemistry and soil technology laboratories, Bureau of Plant Industry, showing storage solutions and plant materials used in the study of natural-color preservation. (Photograph by the division of publications, Department of Agriculture and Commerce.)
2. Some samples of fruits and vegetables preserved in their natural colors. Agricultural chemistry laboratories, Bureau of Plant Industry. (Photograph by the division of publications, Department of Agriculture and Commerce.)
  3. Philippine fruits and vegetables preserved in their natural colors. Bureau of Plant Industry, Department of Agriculture and Commerce.





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2



3

PLATE 1.



## THE PHYSICAL CHARACTERISTICS AND CHEMICAL COMPOSITION OF VARIOUS BRANDS OF TOYO (SOY SAUCE) SOLD IN THE PHILIPPINES <sup>1</sup>

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Toyo, known to Americans and Europeans as soy sauce or soya sauce, or sauce oil, to the Japanese as *shoyu moromi* (Church, 1923), to the Chinese as *ch'au yau*, *pak yau* (Groff, 1919), or *chiang yoo* (Campbell, 1929), is a salty, dark brown liquid, prepared from a specially fermented mixture of cooked soy beans, flour, and salt. To the average Chinese, this sauce is about as important as the rest of the daily diet. Likewise, the Japanese use toyo daily as a condiment or seasoning agent. In Europe and America, soy sauce is known as the basic ingredient of the Worcestershire type of sauce, to which it imparts a characteristic flavor. The use has not been as widespread in the Philippines as in other Oriental countries because there is in this country a counterpart of toyo, the native *patis* <sup>2</sup> (Tag.), but with the growing consciousness of the real nutritive value of the soy bean as a human food, the increasing popularity of the properly made toyo is assured. As sauce imported in the greatest portion into the Philippines from Japan and China is toyo, Table 1, giving the annual imports of sauces into the Philippines as compiled by the Division of Statistics of the Department of Agriculture and Commerce, is of interest as it shows that this sauce is being used in larger and larger quantities.

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<sup>1</sup> Submitted for publication January, 1934.

<sup>2</sup> A high-protein extract from brine-fermented small fish and shrimps.

TABLE 1.—Annual imports of sauces into the Philippines by countries of origin; 1928 to 1933

| Countries of origin.     | 1928      |         | 1929      |         | 1930      |         |
|--------------------------|-----------|---------|-----------|---------|-----------|---------|
|                          | Kilos.    | Pesos.  | Kilos.    | Pesos.  | Kilos.    | Pesos.  |
|                          | Kilos.    | Pesos.  | Kilos.    | Pesos.  | Kilos.    | Pesos.  |
| United States.....       | 544,147   | 251,112 | 500,257   | 219,298 | 475,728   | 184,190 |
| Great Britain.....       | 1,359     | 575     | 610       | 429     | 8,368     | 1,720   |
| France.....              | 452       | 358     | 189       | 274     |           |         |
| Belgium.....             |           |         |           |         |           |         |
| Germany.....             | 689       | 936     | 48        | 28      | 300       | 365     |
| Spain.....               |           |         | 98        | 19      | 56        | 19      |
| Switzerland.....         | 1,781     | 3,531   | 2,344     | 4,873   | 1,661     | 2,956   |
| China.....               | 361,376   | 71,806  | 325,891   | 67,181  | 314,520   | 50,346  |
| British East Indies..... | 360       | 25      | 1,076     | 85      | 1,184     | 120     |
| Dutch East Indies.....   | 7         | 3       |           |         | 310       | 58      |
| Japan.....               | 224,089   | 51,652  | 280,240   | 61,721  | 296,721   | 62,457  |
| French East Indies.....  | 2         | 1       |           |         |           |         |
| Australia.....           | 2         | 2       |           |         |           |         |
| Italy.....               |           |         |           |         | 145       | 63      |
| Netherlands.....         |           |         |           |         |           |         |
| Egypt.....               |           |         |           |         |           |         |
| New Zealand.....         |           |         |           |         |           |         |
| Hongkong.....            |           |         |           |         |           |         |
| Syria.....               |           |         |           |         |           |         |
| Total.....               | 1,134,214 | 379,501 | 1,110,753 | 353,908 | 1,098,993 | 302,294 |

| Countries of origin.     | 1931      |         | 1932      |         | January to November 1933. <sup>a</sup> |         |
|--------------------------|-----------|---------|-----------|---------|----------------------------------------|---------|
|                          | Kilos.    | Pesos.  | Kilos.    | Pesos.  | Kilos.                                 | Pesos.  |
|                          | Kilos.    | Pesos.  | Kilos.    | Pesos.  | Kilos.                                 | Pesos.  |
| United States.....       | 680,222   | 231,610 | 507,843   | 152,598 | 527,501                                | 132,556 |
| Great Britain.....       | 1,129     | 1,210   | 5,319     | 2,586   | 5,114                                  | 3,803   |
| France.....              | 328       | 466     |           |         | 174                                    | 254     |
| Belgium.....             | 20        | 4       |           |         | 13                                     | 3       |
| Germany.....             | 375       | 513     | 189       | 365     | 88                                     | 155     |
| Spain.....               | 733       | 170     |           |         | 70                                     | 382     |
| Switzerland.....         | 3,563     | 6,651   | 4,219     | 5,222   | 3,681                                  | 4,775   |
| China.....               | 710,397   | 72,035  | 1,099,883 | 98,026  | 635,724                                | 75,662  |
| British East Indies..... | 1,111     | 147     | 2,012     | 257     | 1,041                                  | 76      |
| Dutch East Indies.....   | 9         | 2       | 4         | 2       |                                        |         |
| Japan.....               | 308,781   | 55,711  | 341,680   | 36,567  | 273,158                                | 36,315  |
| French East Indies.....  |           |         |           |         |                                        |         |
| Australia.....           | 316       | 152     | 934       | 218     | 92                                     | 38      |
| Italy.....               |           |         | 308       | 85      | 123                                    | 74      |
| Netherlands.....         | 15        | 10      | 1         | 1       | 3                                      | 1       |
| Egypt.....               | 30        | 6       |           |         |                                        |         |
| New Zealand.....         |           |         |           |         | 4                                      | 2       |
| Hongkong.....            |           |         | 567       | 35      | 35,287                                 | 5,050   |
| Syria.....               |           |         | 13        | 4       |                                        |         |
| Total.....               | 1,707,029 | 368,687 | 1,962,972 | 295,966 | 1,482,073                              | 259,146 |

<sup>a</sup> January to November only, December figures not available.

The value of soy sauce as an article of food lies in its nutritive seasoning qualities. The main reason, of course, is because it is a product of soy beans, which are very rich in good-quality proteins, fats, and minerals. Table 2, compiled from analyses of beans, shows the proximate chemical composition of soy and other beans raised in the Philippines (Adriano et al., 1925, 1928, 1929, 1931, and 1932).

TABLE 2.—*Proximate chemical composition of soy and other Philippine beans.*

| Sample.                         | Moisture.     | Ash.          | Dry basis.           |                |                  |          |                                       |
|---------------------------------|---------------|---------------|----------------------|----------------|------------------|----------|---------------------------------------|
|                                 |               |               | Protein<br>N x 6.25. | Crude<br>fats. | Crude<br>fibers. | N. F. E. | Food<br>value,<br>calories<br>per kg. |
|                                 | <i>P. ct.</i> | <i>P. ct.</i> | <i>P. ct.</i>        | <i>P. ct.</i>  | <i>P. ct.</i>    |          |                                       |
| Common beans (habichuelas)----- | 10.77         | 4.62          | 22.94                | 2.27           | 1.75             | 68.32    | 3,527                                 |
| Kidney beans-----               | 9.80          | 4.66          | 22.59                | 7.50           | 4.06             | 61.18    | 3,727                                 |
| Lima beans-----                 | 12.56         | 4.55          | 18.56                | 2.90           | 2.39             | 71.97    | 3,481                                 |
| Mongo beans-----                | 12.41         | 4.53          | 23.71                | 1.68           | 4.23             | 65.91    | 3,359                                 |
| Soy beans-----                  | 12.78         | 5.76          | 39.50                | 19.25          | 9.35             | 24.06    | 3,834                                 |

A review of analyses of soy beans reported in literature shows the following variations according to Gibbs and Agcaoili (1912): water, 9 to 13 per cent; proteins, 31 to 42 per cent; fat, 14 to 22 per cent; nitrogen-free extract, 27 to 31 per cent; crude fiber, 4 to 13 per cent; starch, trace to 3.5 per cent; ash, 4.7 to 5.9 per cent. The analyses cited by the same authors show that 85 to 90 per cent of nitrogenous substance in the soy bean is pure protein and 10 to 15 per cent nonprotein; that the chief protein material is glycinin, a globulin precipitable by lime salts, the precipitate being soluble in sodium chloride solutions; and that starch is present in small quantities only, but that the sugars amount to 12 per cent. According to Horvath (1933), the oil in soy beans can be assimilated completely in normal doses.

Another reason for the extensive use of toyo is its high flavoring and appetite-stimulating properties. Therefore, it is widely used among peoples whose diet is mainly composed of starchy foods, or among those whose meals, due to pecuniary circumstances, tend to become monotonous.

#### PRINCIPLES INVOLVED IN TOYO MANUFACTURE

The underlying principle in the manufacture of toyo is the hydrolysis of the proteins of the soy beans and of the proteins

of accessory substances. These accessory substances consist largely in starch-bearing products to supplement the starch deficiency of the soy bean. Generally wheat, either ground roasted, bran, or commercial flour, is used. For hydrolysis, proteolytic and diastatic enzymes, produced during the vegetative growth of certain molds, particularly of the *Aspergillus flavus-oryzæ* group, are used. To meet industrial demands, the mold is grown upon cooked soy beans coated with flour. The flour provides the carbohydrate necessary to stimulate mold growth and the mechanical separation between the individual wet beans in order to expose as large a surface as possible to the mycelia of the mold. The enzymes produced during mold growth act upon the proteins and other food constituents of the mixture. As a preventive against bacterial action, the fermentation or enzyme action is allowed to proceed with the whole mixture submerged in an antiseptic, the commercial antiseptic generally used being brine from ordinary sea or rock salt.

The conversion of the soy beans proteins and added carbohydrates into soluble and simpler forms follows a definite velocity curve, the velocity becoming lessened with time. In commercial practice, however, complete or almost complete hydrolysis of the proteins is not allowed. The duration in which the moldy mixture is set to fermentation is in most cases determined more by economic considerations than by scientific practice. When the aqueous solution thus formed is too dilute, it is generally boiled, at the same time effecting sterilization. Properly brewed, however, the prepared toyo needs no further concentration.

#### RAW MATERIALS

1. *Soy beans*.—It is of course desirable that the soy beans used in the manufacture of toyo should have a high protein and fat content. A moderate amount of carbohydrates, especially starch, is also desirable, since for balanced working, this condition means that less flour needs to be added to the soy bean for molding. The factories in and around Manila, from which most of the soy sauce sold in the Philippines comes, use soy beans that are generally imported from China, the yellow variety being the most commonly used. However, there are now grown in the Philippines soy beans that are very suitable for toyo manufacture. A variety grown by the Bureau of Plant Industry at the central station at San Andres, for example, analyzed, 6.27 ash, 36.50 protein, 21.91 crude fats, 2.15 crude fiber, and 5.96 nitrogen-free extract, according to determinations made by this

laboratory. The Chinese prefer thick-coated beans which remain intact after boiling soft (Groff, 1919).

2. *Starch-bearing material*.—Church (1923) states that the wheat variety chosen as most closely resembling that used in Japan for toyo manufacture is the Stoner variety grown in the Eastern States. This wheat is rich in starch. Among local manufacturers ordinary wheat flour is used. Practice seems to indicate that any starch-bearing material may be used. It is asserted that the use of ground roasted wheat adds a certain flavor to the finished product, but with the low prices prevailing in the local markets, this practice does not seem feasible.

3. *Salt*.—In local factories ordinary salt is used. The salts of calcium and magnesium, other than the basic salts, do not seem to interfere with the fermentation, since the sea salt used contains appreciable quantities of calcium and magnesium, and quite often satisfactory products are turned out. The same is true for other foreign substances present in sea salt.

4. *Mold culture*.—The mold culture used is invariably the yellowish green mold belonging to the *Aspergillus flavus-oryzæ* group. In local factories, while no definite pure cultures are used, the molding room is used exclusively for this purpose only and as the mold reproduces by means of spores, predominance of the spores of this type of organism more nearly approximates the use of pure cultures.

5. *Water*.—Since the manufacture of toyo is a fermentation process in an aqueous medium, it is necessary that the water used should be biologically pure and free from substances that would counteract or in any way inhibit the action of the enzymes used. Foreign bacteria that readily develop in aqueous media often produce bad flavor.

## EQUIPMENT

1. *Equipment for boiling beans*.—The general equipment for boiling beans consists of an open boiler, made of cast iron, set in brickwork which houses the oven. Where fuel and labor costs are low, this system works out well, otherwise it seems preferable to use standard pressure cookers utilizing steam, so that very little of the water-soluble constituents of the soy beans should go to the water used for boiling which is drained off. Semifactory tests have already shown that pressure cooking at 15 pounds steam pressure for fifty minutes softens the beans to the desired point, whereas boiling at ordinary pressure usually requires from five to ten hours.

2. *Molding room*.—The molding room is generally a dark room in which the temperature, ventilation, and humidity can more or less be controlled. After a room has been used for several weeks, a new mold room is well inoculated with the spores of the desired mold. Cooked beans are heaped on trays and these trays are placed in racks in the mold room for mold growth.

3. *Fermenting vats*.—Local factories use large earthenware jars with conical, woven bamboo, covers. These jars are left in the open for sunning. The same method, according to information gathered from the local Chinese manufacturers, and from photographs (Church, 1923), is used in China. In Japan, where there are toyo factories operating on a large scale, the fermenting vats are made of wood or concrete, built or set in regular fermentation rooms.

#### METHOD OF MANUFACTURE

The process of manufacture may be briefly summarized as follows: Cooking the beans, adding the requisite amount of starch-bearing material, inoculating with the specific mold, molding, fermentation or ripening, drawing off, and bottling.

1. *Cooking the beans*.—The beans are cooked till they are soft and can be crushed with the fingers, but not mashed. Ordinarily, the beans are soaked in water for preliminary softening, about twelve to thirty-six hours, after which they are cooked in boiling water until soft. Soaking beans causes some fermentation, the resulting product being sour even after cooking. The defect is remedied to some extent by soaking in several changes of water, and no souring ensues if the soaking is in running water. After the beans have been cooked the excess water is drained off. Present laboratory tests indicate that differential growth or predominance between the desired yellow-green mold and the black mold is due to a great extent to the moisture content of the medium. The draining is done by heaping the cooked beans upon bamboo baskets, one kind of which is the native *kaing* or *tiklis* (Tag.).

During the draining, the beans gradually cool. When no more water drips off, the beans are ready to be coated with the starch-bearing material.

The effect of cooking upon the fermentation is clearly seen from the fact that a hard product causes slow action of the enzymes. It is possible that the penetration of the mycelia of

the mold into the cooked beans influences the mode of fermentation. It is also obvious that the mycelia cannot penetrate with equal facility into hard substrata as into soft substrata.

2. *Coating with starch.*—Commercial wheat flour is generally used. Tests by the United States Department of Agriculture showed that the best proportion of ground crushed wheat to the cooked soy bean was between one part wheat to three parts cooked beans by weight, to two to three. The coating of starch prevents the packing of the cooked beans—a condition that generally induces bacterial fermentation and souring. It also increases the effective surface of the soy beans for the growth of the mold. Needless to say that the greater the mold growth, the greater the quantity of enzymes produced under equal conditions.

A large excess of starch over that required by the mold not only entails more expense, but also carries into the final residue whatever starch is left undigested. Or if the final toyo be sufficiently thick or viscous to carry the starch particles in suspension, the resulting product is turbid, and upon subjection to heat, precipitates a fine sediment.

3. *Inoculation and molding.*—Japanese manufacturers generally inoculate fresh material with some well-molded material to insure proper growth. As stated, the mold room is generally full of potent spores of the organism, making inoculation unnecessary. In the fermentation industries, however, it is the accepted practice to inoculate, and not to depend upon natural inoculation, as this method leaves too much of the process to chance. The mold is easy to cultivate, detect, and multiply. The quantity of inoculum should be such that there should be no more than the necessary mold growth, because an excess of mold life means the using up of more material to maintain mold growth.

The inoculated mass is spread evenly upon the trays, to a depth of about one inch. In the native factories, the trays are the common wide, shallow bamboo baskets called *bilao* (Tag.). Since mold growth in toyo manufacture is accompanied by the rise of temperature of the mass and since too high a temperature is not conducive to the proper formation of the desired enzymes, but induces undesirable chemical reactions in the mass as evidenced by the liberation of an undesirable odor (Harada, 1931; Church, 1923), it is essential to dissipate this heat. The mold rooms of local toyo factories are either open to the rest of the

building, or else have wide windows which can be opened when necessary.

The molding mass is not stirred in the local factories, although it is obvious that to obtain uniform growth, some sort stirring, or exposing the surface of the cooked beans is necessary. With proper mold growth, the coated beans form a solid cake, the individual particles of which are evenly coated with the mycelia of the organism. Foreign growths are undesirable since they produce bad flavor in the resulting product.

Mold growth is stopped when sporulation takes place, that is, when the surface of the mass is coated with yellow spores. Too heavy sporulation is avoided. While local factories terminate mold growth after seven to fifteen days, it is possible to have all the necessary mold growth in two to three days.

4. *Brewing, fermenting or ripening.*—The moldy material is dumped into the fermenting vats and covered with brine of 20 to 22 per cent concentration. Here the enzymes act upon the food substances in the soy bean and wheat. The brine prevents, to a certain extent, the growth of foreign and undesirable organisms. The Chinese, like the local manufacturers, sun the brine suspension, while the Japanese keep the suspension in properly heated fermentation cellars.

After a certain period of time when it is judged that the enzymic action has proceeded far enough, the clear liquid is separated from the rest of the mass. In the local factories, after sunning for two to four months, the brine concentration in the fermenting vats has increased to such a point that part of the salt crystallizes out. Before drawing off the sauce, therefore, the fermented mixture is treated with a more dilute salt solution. The brown liquid is then siphoned off with a foot valve made out of bamboo at the intake end of the siphon. The drawn sauce may be boiled, but generally, it is bottled without heat treatment to be sold as first-class toyo. The residue is brined and sunned again to obtain succeeding lower grades of toyo. In Japan (Church, 1923), the system of multiple drawing off is not practiced; instead, the ripe sauce "moromi" is pressed to obtain the sauce. No heat treatment follows.

#### SAMPLES ANALYZED

Table 3 shows the samples analyzed in this investigation. The names and addresses of the manufacturers and trade marks of the products are included. One sample came from Japan, two samples were manufactured in China, and the rest were

manufactured in and around the suburbs of Manila. One sample is canned, while the rest are bottled. All samples were thoroughly mixed by shaking before taking samples for analysis.

TABLE 3.—*Samples of toyo analyzed.*

| Sample No. | Brand.                                                   | Name.                                 | Address.                                 |
|------------|----------------------------------------------------------|---------------------------------------|------------------------------------------|
| 1          | Imitation Worcester-shire—Volcano.                       | Chan Ching Co.....                    | 514 Juan Luna, Manila.                   |
| 2          | Dos Banderas Primera                                     | do.....                               | Do.                                      |
| 3          | Violin Segunda                                           | Philippine Products Manufacturing Co. | 225 Otis, Paco, Manila.                  |
| 4          | Dollar, 3d class                                         | do.....                               | Do.                                      |
| 5          | Carrera                                                  | Chan Ching Co.....                    | 13 Riverside, San Juan del Monte, Rizal. |
| 6          | Pavo Primera                                             | Kim Chuan Soy Factory                 | 1403 La Loma, Caloocan, Rizal.           |
| 7          | Boy Scout, 2d class                                      | do.....                               | Do.                                      |
| 8          | Un Caballo Primera                                       | Chan Ching Co.....                    | 514 Juan Luna, Manila.                   |
| 9          | Cock                                                     | Kwong Shing Yuen Chinese Sauce Mfg.   | 727 Almanza, Manila.                     |
| 10         | Goose—red label                                          | The Amoy Canning Corp., Ltd.          | Amoy, China.                             |
| 11         | Toyo—Chinese saucc; Imitation Worcester sauce—1st class. | Kwong Shing Yuen                      | 727 Azcarraga, Manila.                   |
| 12         | The Kikkoman Shoyu                                       | Nedashoyu Co., Ltd.                   | Japan.                                   |
| 13         | Gallo                                                    | Kwong Shing Yuen                      | 727 Almanza, Manila.                     |
| 14         | Violin Primera                                           | Philippine Products Manufacturing Co. | 225 Otis, Paco, Manila.                  |
| 15         | Dollar, 2d class                                         | do.....                               | Do.                                      |
| 16         | Sang Yiek                                                |                                       | Manila.                                  |
| 17         | Pineapple soy, 1st class                                 | Kwong Me Chan                         | 440 T. Pinpin, Manila.                   |
| 18         | Goose, big label                                         | Amoy Chinese Canning factory.         | Amoy, China.                             |
| 19         | Sunrise                                                  | Sengkee Company                       | Nueva, Manila.                           |
| 20         | Butterfly Superior Soy                                   | Kwong Me Chan                         | 440 T. Pinpin, Manila.                   |
| 21         | Bell soy                                                 |                                       | 1361 La Loma, Caloocan, Rizal.           |

## METHODS OF ANALYSIS USED

For the most part the Official and Tentative Methods of Analysis (1930) were followed. Modified methods of Adriano (1932) as well as those reported by Leach (1913) were also adopted. Only common terms were used in describing taste and odor. The samples were tested at one time and the comparative taste and odor noted. As the samples were too dark for optical observation, 10 cc were diluted to 500 cc with distilled water, and the diluted solution observed for color and clarity.



| Sample No. | Moisture. | Total solids. | Brix.    | pH.  | Total ash. | Proteins<br>N x 6.25. | Fats.  | Reducing<br>sugars. | Sucrose. | Starch. | Sodium<br>chloride. | Total so-<br>lids not<br>ash. | Ash not<br>sodium<br>chloride. |
|------------|-----------|---------------|----------|------|------------|-----------------------|--------|---------------------|----------|---------|---------------------|-------------------------------|--------------------------------|
|            | P. ct.    | P. ct.        | Degrees. |      | P. ct.     | P. ct.                | P. ct. | P. ct.              | P. ct.   | P. ct.  | P. ct.              | P. ct.                        | P. ct.                         |
| 1          | 70.96     | 29.04         | 47       | 4.4  | 18.52      | 4.14                  | 0.49   | 10.09               | 0.00     | 0.00    | 17.87               | 10.52                         | 0.65                           |
| 2          | 63.42     | 36.58         | 58.4     | 4.9  | 21.34      | 3.46                  | 1.44   | 12.37               | 0.72     | 0.00    | 19.63               | 15.24                         | 1.71                           |
| 3          | 62.53     | 37.47         | 56.3     | 5.6  | 23.44      | 2.26                  | 1.06   | 7.57                | 1.82     | 2.43    | 21.36               | 14.03                         | 2.08                           |
| 4          | 63.30     | 36.69         | 56.0     | 5.5  | 22.39      | 1.58                  | 0.59   | 8.28                | 0.00     | 1.96    | 20.51               | 14.30                         | 1.88                           |
| 5          | 63.99     | 35.61         | 55       | 5.3  | 21.89      | 1.71                  | 1.65   | 9.60                | 1.39     | 0.95    | 19.74               | 13.72                         | 2.15                           |
| 6          | 53.96     | 46.04         | 60.7     | 5.6  | 21.04      | 13.31                 | 1.27   | 5.55                | 0.00     | 0.51    | 19.23               | 25.00                         | 1.81                           |
| 7          | 56.58     | 43.42         | 60.2     | 5.6  | 21.45      | 13.37                 | 2.05   | 5.50                | 0.43     | 0.00    | 19.52               | 21.97                         | 1.93                           |
| 8          | 61.47     | 38.53         | 58.8     | 5.4  | 22.29      | 3.52                  | 1.94   | 12.12               | 1.50     | 1.38    | 20.18               | 16.24                         | 2.11                           |
| 9          | 65.79     | 33.21         | 51.1     | 5.45 | 24.23      | 4.05                  | 0.99   | 3.91                | 0.11     | 0.00    | 21.09               | 38.98                         | 3.14                           |
| 10         | 63.01     | 36.99         | 52.5     | 5.45 | 23.62      | 8.29                  | 1.20   | 12.00               | 0.00     | 0.00    | 21.47               | 13.29                         | 2.15                           |
| 11         | 62.17     | 37.83         | 58.8     | 6.05 | 21.30      | 2.39                  | 0.15   | 12.58               | 1.39     | 3.94    | 19.16               | 16.53                         | 2.14                           |
| 12         | 68.72     | 31.28         | 43.8     | 6.35 | 17.11      | 7.93                  | 2.54   | 2.86                | 0.96     | 0.00    | 15.25               | 14.17                         | 1.85                           |
| 13         | 59.15     | 40.85         | 57.8     | 5.9  | 20.05      | 3.32                  | 1.61   | 12.90               | 0.00     | 2.81    | 18.59               | 20.80                         | 1.46                           |
| 14         | 63.34     | 36.66         | 61.2     | 6.3  | 19.98      | 4.47                  | 1.32   | 9.20                | 0.00     | 0.00    | 17.38               | 16.68                         | 2.60                           |
| 15         | 62.99     | 37.01         | 58.8     | 6.2  | 22.66      | 2.76                  | 1.48   | 8.16                | 0.00     | 0.00    | 20.26               | 14.35                         | 2.40                           |
| 16         | 61.28     | 38.72         | 53.7     | 5.45 | 21.81      | 3.76                  | 1.61   | 13.28               | 0.45     | 0.39    | 19.57               | 16.98                         | 2.24                           |
| 17         | 59.51     | 40.49         | 56.5     | 5.85 | 22.96      | 3.94                  | 2.19   | 10.33               | 1.06     | 0.42    | 20.66               | 17.53                         | 2.30                           |
| 18         | 65.49     | 34.51         | 51.9     | 5.45 | 22.71      | 2.61                  | 1.09   | 5.52                | 0.47     | 0.00    | 20.43               | 11.80                         | 2.28                           |
| 19         | 66.97     | 33.03         | 44.7     | 5.7  | 23.98      | 4.85                  | 1.54   | 7.66                | 0.53     | 1.98    | 21.85               | 9.05                          | 2.13                           |
| 20         | 60.24     | 39.76         | 57.1     | 5.35 | 28.18      | 1.60                  | 0.93   | 9.82                | 1.08     | 0.64    | 24.62               | 11.58                         | 3.56                           |
| 21         | 61.21     | 38.79         | 61.2     | 5.2  | 21.47      | 2.51                  | 0.90   | 15.36               | 0.60     | 1.60    | 19.48               | 17.32                         | 1.99                           |
| Average    | 62.69     | 37.31         | 55.31    | 5.6  | 22.02      | 4.56                  | 1.33   | 9.27                | 0.59     | 0.90    | 19.90               | 17.32                         | 2.12                           |

<sup>b</sup> Can.<sup>a</sup> Computed

TABLE 5.—Physical characteristics and chemical composition of various brands of toyo (soy sauce) sold in the Philippines.  
Analyses on moisture-free basis.

| Sample No. | Total solids. |        | Total ash. |        | Proteins<br>N x 6.25. |        | Ether<br>extract. |        | Reducing<br>sugars. |        | Sucrose. |        | Starch. |        | Sodium<br>chloride. |        | Total so-<br>lids not<br>sodium<br>chloride. |        | Ash not<br>sodium<br>chloride. |  |
|------------|---------------|--------|------------|--------|-----------------------|--------|-------------------|--------|---------------------|--------|----------|--------|---------|--------|---------------------|--------|----------------------------------------------|--------|--------------------------------|--|
|            | P. ct.        | P. ct. | P. ct.     | P. ct. | P. ct.                | P. ct. | P. ct.            | P. ct. | P. ct.              | P. ct. | P. ct.   | P. ct. | P. ct.  | P. ct. | P. ct.              | P. ct. | P. ct.                                       | P. ct. | P. ct.                         |  |
| 1          | 29.04         | 63.77  | 14.26      | 1.69   | 34.74                 | 0.00   | 0.00              | 0.00   | 34.74               | 0.00   | 0.00     | 61.53  | 38.47   | 2.24   | 61.53               | 38.47  | 2.24                                         | 2.24   | 2.24                           |  |
| 2          | 36.58         | 58.34  | 9.46       | 3.94   | 33.82                 | 1.97   | 0.00              | 3.94   | 33.82               | 1.97   | 0.00     | 53.66  | 46.34   | 4.68   | 53.66               | 46.34  | 4.68                                         | 4.68   | 4.68                           |  |
| 3          | 37.47         | 62.56  | 6.03       | 2.83   | 20.20                 | 4.86   | 6.48              | 2.83   | 20.20               | 4.86   | 6.48     | 57.01  | 42.99   | 5.55   | 57.01               | 42.99  | 5.55                                         | 5.55   | 5.55                           |  |
| 4          | 36.69         | 61.02  | 4.31       | 1.61   | 22.57                 | 0.00   | 5.34              | 1.61   | 22.57               | 0.00   | 5.34     | 55.90  | 44.10   | 5.12   | 55.90               | 44.10  | 5.12                                         | 5.12   | 5.12                           |  |
| 5          | 35.61         | 61.47  | 4.80       | 4.63   | 26.96                 | 3.90   | 2.67              | 4.63   | 26.96               | 3.90   | 2.67     | 55.43  | 44.57   | 6.04   | 55.43               | 44.57  | 6.04                                         | 6.04   | 6.04                           |  |
| 6          | 46.04         | 45.69  | 28.91      | 2.76   | 12.05                 | 0.00   | 1.11              | 2.76   | 12.05               | 0.00   | 1.11     | 41.77  | 58.23   | 3.92   | 41.77               | 58.23  | 3.92                                         | 3.92   | 3.92                           |  |
| 7          | 43.42         | 49.40  | 30.79      | 4.72   | 12.57                 | 0.99   | 0.00              | 4.72   | 12.57               | 0.99   | 0.00     | 44.96  | 55.04   | 4.44   | 44.96               | 55.04  | 4.44                                         | 4.44   | 4.44                           |  |
| 8          | 38.53         | 57.85  | 9.06       | 4.99   | 31.45                 | 3.89   | 3.58              | 4.99   | 31.45               | 3.89   | 3.58     | 52.37  | 47.63   | 5.48   | 52.37               | 47.63  | 5.48                                         | 5.48   | 5.48                           |  |
| 9          | 38.98         | 72.96  | 12.19      | 2.98   | 11.77                 | 9.33   | 0.00              | 2.98   | 11.77               | 9.33   | 0.00     | 63.50  | 36.50   | 9.46   | 63.50               | 36.50  | 9.46                                         | 9.46   | 9.46                           |  |
| 10         | 36.99         | 63.99  | 22.46      | 3.25   | 32.51                 | 0.00   | 0.00              | 3.25   | 32.51               | 0.00   | 0.00     | 58.17  | 41.83   | 5.82   | 58.17               | 41.83  | 5.82                                         | 5.82   | 5.82                           |  |
| 11         | 37.83         | 56.30  | 6.32       | 0.40   | 33.25                 | 3.67   | 10.41             | 0.40   | 33.25               | 3.67   | 10.41    | 50.65  | 49.35   | 5.65   | 50.65               | 49.35  | 5.65                                         | 5.65   | 5.65                           |  |
| 12         | 31.28         | 54.70  | 25.35      | 8.12   | 9.14                  | 3.07   | 0.00              | 8.12   | 9.14                | 3.07   | 0.00     | 48.75  | 51.25   | 5.95   | 48.75               | 51.25  | 5.95                                         | 5.95   | 5.95                           |  |
| 13         | 40.85         | 49.08  | 8.13       | 3.94   | 31.58                 | 0.00   | 6.88              | 3.94   | 31.58               | 0.00   | 6.88     | 45.50  | 54.50   | 3.58   | 45.50               | 54.50  | 3.58                                         | 3.58   | 3.58                           |  |
| 14         | 36.66         | 54.50  | 12.19      | 3.60   | 25.09                 | 0.00   | 0.00              | 3.60   | 25.09               | 0.00   | 0.00     | 47.40  | 52.60   | 7.10   | 47.40               | 52.60  | 7.10                                         | 7.10   | 7.10                           |  |
| 15         | 37.01         | 61.22  | 7.45       | 2.46   | 22.05                 | 0.00   | 0.00              | 2.46   | 22.05               | 0.00   | 0.00     | 54.74  | 45.26   | 6.48   | 54.74               | 45.26  | 6.48                                         | 6.48   | 6.48                           |  |
| 16         | 38.72         | 56.33  | 9.71       | 4.16   | 34.30                 | 1.16   | 1.01              | 4.16   | 34.30               | 1.16   | 1.01     | 50.54  | 49.46   | 5.79   | 50.54               | 49.46  | 5.79                                         | 5.79   | 5.79                           |  |
| 17         | 40.49         | 56.70  | 9.73       | 5.40   | 25.51                 | 2.62   | 1.04              | 5.40   | 25.51               | 2.62   | 1.04     | 51.02  | 48.98   | 5.68   | 51.02               | 48.98  | 5.68                                         | 5.68   | 5.68                           |  |
| 18         | 34.51         | 65.81  | 7.56       | 3.16   | 15.99                 | 1.36   | 0.00              | 3.16   | 15.99               | 1.36   | 0.00     | 59.20  | 40.80   | 6.61   | 59.20               | 40.80  | 6.61                                         | 6.61   | 6.61                           |  |
| 19         | 33.03         | 72.60  | 14.68      | 4.66   | 23.19                 | 1.60   | 5.99              | 4.66   | 23.19               | 1.60   | 5.99     | 66.15  | 33.85   | 6.45   | 66.15               | 33.85  | 6.45                                         | 6.45   | 6.45                           |  |
| 20         | 39.76         | 70.87  | 4.02       | 2.34   | 24.69                 | 2.71   | 1.61              | 2.34   | 24.69               | 2.71   | 1.61     | 61.92  | 38.08   | 8.95   | 61.92               | 38.08  | 8.95                                         | 8.95   | 8.95                           |  |
| 21         | 38.79         | 55.39  | 6.47       | 2.32   | 39.63                 | 1.55   | 4.13              | 2.32   | 39.63               | 1.55   | 4.13     | 50.26  | 49.74   | 5.13   | 50.26               | 49.74  | 5.13                                         | 5.13   | 5.13                           |  |
| Average    | 37.31         | 59.55  | 12.09      | 3.52   | 24.15                 | 1.65   | 2.39              | 3.52   | 24.15               | 1.65   | 2.39     | 53.83  | 46.17   | 5.72   | 53.83               | 46.17  | 5.72                                         | 5.72   | 5.72                           |  |

## RESULTS AND DISCUSSION

Tables 4 and 5 show the physical characteristics and chemical composition of toyo (soy sauce) sold in the Philippines. Of the twenty-one samples analyzed, one sample was packed in a 200-cc bottle, one in a 250-cc bottle, one in a 300-cc, six in 325-cc bottles, nine in 500-cc bottles, two in 650-cc bottles, and one in a 1,500-cc tin can. The computed cost per liter varied from 28 centavos to 1.33 pesos. All the samples tasted fair to good. The odor was generally from fair to very good. The specific gravity varied from 1.975 to 1.3045.

The moisture content varied from 53.96 to 70.96 per cent, giving a total-solids content varying from 29.04 to 46.04 per cent. The total solids consist of ash, varying from 17.11 to 28.18 per cent, of which the sodium chloride varied from 15.25 to 24.62 per cent; proteins, varying from 1.58 to 13.37 per cent; fats or ether extract, varying from 0.15 to 2.54 per cent; reducing sugars, varying from 2.86 to 15.36 per cent; sucrose, from 0.0 to 1.82 per cent; and starch from 0.0 to 3.94 per cent. The difference between the ash and the sodium chloride percentages varied from 0.65 to 3.56 per cent.

If the average moisture content of soy beans is taken to be 11 per cent, and the average total proteins 36 per cent, 10 per cent of which is convertible to soluble and simpler forms, and if the efficiency of fermentation be 80 per cent, 29 per cent of the total solids should be the protein matter in the toyo. If, from the average of the determinations reported here, the total solids in toyo be 37 per cent, the protein content of the finished product should be not less than 7.5 per cent. Using this arbitrary standard, it may be seen from Table 3 that only four of the twenty-one samples analyzed contain the required percentage of proteins. An explanation of the usually low protein content may be that locally manufactured toyo is sunned, the temperature quite often reaching nearly 45° C. At this temperature, Harada (1931) has shown that the diastatic activity is decreased, even stopped. Also that the optimum pH for enzymic activity approaches neutrality, which is hard to obtain in the mash. Therefore the rate of hydrolysis of the food substances in the mash is decreased. Another reason is the system of multiple drawings. After a certain period, which should be as short as possible in order not to tie up capital unnecessarily, toyo is siphoned off, even if the protein content is still low. Since the demand does not quite dictate a better

product, the practice of admixing molasses is widespread. The use of molasses as a diluent may also account for the low protein content as well as for the production of an inferior product.

An interesting constituent of toyo is the reducing sugar. Of the twenty-one samples analyzed, twelve contained reducing sugars analyzing about 10 per cent, and only two contained below 5 per cent. Harada (1931), from a survey of the literature, states that the enzymes formed by the *Aspergillus* mold used in the manufacture of toyo include invertase, maltase, and dextrinase. The presence of reducing sugars is partially due to the action of these enzymes upon the sucrose which results from the hydrolysis of the starch. It is believed, however, that part of the reducing sugars amounting to 10 per cent or more is due to the addition of molasses or caramel as adulterant, which is a practice very common among the local manufacturers, the drawn off toyo being boiled with molasses to the desired consistency.

One sample coming from Japan, with an ash content lower than 18 per cent, and ash-not-sodium chloride percentage of 15.25, was not as salty as the other samples analyzed. Many of the samples analyzed have very high salt contents, hence some solid salt crystallizes out in the bottle, probably due to bottling the hot, salt-saturated toyo. The difference between the total ash and the sodium chloride may be taken as making up the other minerals, such as calcium and magnesium.

The fat or ether extract content of the samples analyzed was in general low. The average percentage of fat in the toyo samples analyzed represents only about one-eighth of the total fat in the soy bean. Taking the sample analyzing the highest fat, it is possible to have in the toyo about one-half of the fat present in the soy bean, calculated on the dry basis. Pending further study, this probability may be used as a basis for setting standards for market classes of the sauce. The generally low fat content in the finished products may be due to the factors discussed under the low content of proteins.

The sucrose in toyo probably comes from saccharine adulterants such as molasses. In fifteen of twenty-one samples analyzed sucrose was present, the lowest being 0.11 per cent and the highest, 1.82 per cent. It is believed, however, that the sucrose content depends upon the period of storage, because even if the sucrose were high to start with, the pH of the samples precludes the long existence of high percentages of sucrose in the toyo after some time.

The total solids in toyo have a high content of sodium chloride. They cannot therefore be taken as an index upon which to base toyo standards. On a moisture-free basis the total solids-not-ash varies from 33.85 to 58.23 per cent, the average being 45.17 per cent. These values represent mostly food elements in the toyo except the minerals.

The portion of ash that is not sodium chloride varies from 2.24 to 9.46 per cent on a moisture-free basis, with an average of 5.72 per cent. The mineral matter of toyo comes from the ash of the soy beans and starch-bearing material, and also, for the most part, from the salt used.

#### SUMMARY

Twenty-one samples of toyo—one from Japan, two from China, and eighteen from Philippine factories—were analyzed.

The computed cost ranged from 31 centavos to 1.33 pesos per liter. The taste of the samples was either fair or good, but the odor ranged from poor to very good. Upon diluting fifty times with distilled water, the samples were generally clear brown, five samples, however, were cloudy or turbid.

The specific gravity at 25° C. varied from 1.1975 to 1.3045, the moisture from 53.96 to 70.96 per cent, the pH from 4.4 to 6.35 per cent, the total ash from 17.11 to 28.18 per cent, and the sodium chloride from 15.25 to 24.62 per cent.

Of the other food constituents the proteins varied from 1.58 to 13.37 per cent, the fats from 0.49 to 2.54 per cent, the reducing sugars from 2.86 to 15.36 per cent, the sucrose from 0.0 to 1.82, and the starch from 0.0 to 3.94 per cent.

It is believed that standards for toyo in the market should take into consideration not only the protein content, but also the fat, reducing sugars, and total solids-not-ash percentage.

According to Dr. A. J. Hermano, of the Bureau of Science, toyo (soy sauce) should meet the following standards:

Specific gravity. 1.23 to 1.25.

Total solids. Not less than 26 g per 100 ml sample.

Proteins. Not less than 4.5 per cent.

Alkalinity of ash. Not more than 1.5 ml 0.1N standard acid solution per g.

Comparing with these standards, we find that nineteen of the twenty-one samples analyzed come up to the specifications for specific gravity, all contain the required total solids, and only six meet the requirement for proteins. Of these six, one is below specification for specific gravity.

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HON. EULOGIO RODRIGUEZ  
Secretary of Agriculture and Commerce

*[See biographical sketch on p. 187]*

## Quarterly Notes

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### OUR NEW SECRETARY

A successful business man and farmer and a politician of no mean ability—such in a nutshell is the colorful career of the Hon. Eulogio Rodriguez who in July was appointed Secretary of Agriculture and Commerce. His early struggles for an economic foothold and his determination to make a name for himself by sheer merit would constitute an appropriate plot in a success story, and he himself would make an appropriate hero.

Secretary Rodriguez was born on March 21, 1883, in Montalban, Rizal. His parents, Petronilo Rodriguez and Monica Adona, were not of the rich class but gave all opportunities they could to young Eulogio, by sending him first to a primary school of the town, and later to the Colegio de San Juan de Letran. From this College he obtained his bachelor of arts degree. He then took up law, completed the course, and later was admitted to the Philippine bar.

As is usually the case with a fresh practitioner in the legal profession, he found that his practice was not lucrative and became discouraged. Having a natural inclination for business, he turned his attention and efforts to that. His inability to make money in the legal profession proved to be a blessing in disguise, for instead of remaining a mediocre lawyer plodding along with what few cases came his way, he became a real asset to his country as a successful business man. After abandoning his profession he occupied himself with what little money he could command with supplying fodder and firewood to American soldiers stationed in Marikina and its environs during the early days of the American régime. Later he established four saloon bars that catered to the American soldiers. His business soon prospered and he became a contractor on a bigger scale. With the profit he made of his business he branched out into farming by buying lands in the province and developing them.

Soon thereafter he established himself in Manila, where in 1919 he organized with some leading business men, the Luzon

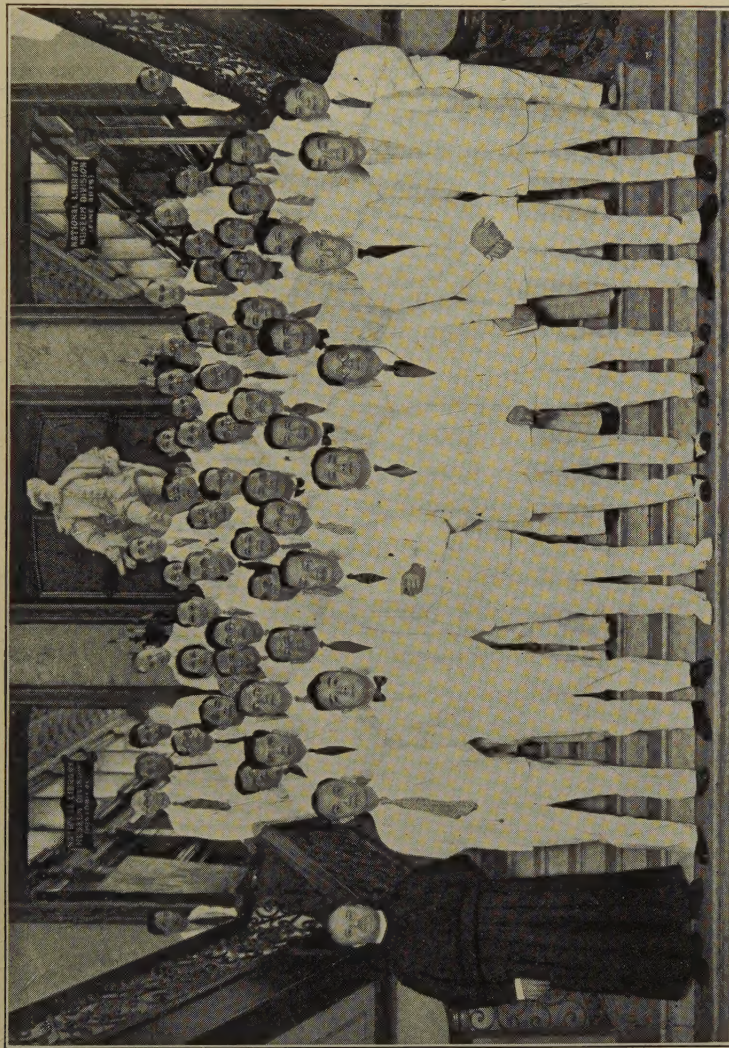
Surety Company, of which he later became manager. At the time of his appointment he was director of several corporations and president of the Philippine Chamber of Commerce. When he accepted the secretaryship, he resigned from all the offices he was holding in the different firms he was connected with.

As a sideline he went into politics in 1909 and met with success, being elected municipal president of Marikina. In 1912 he was reelected. When his term expired in 1916, he ran for governor of Rizal, was elected and twice reelected to this office. In July, 1923, he was named Mayor of Manila, a position he held only for a few months. Later, in 1924, he was appointed Representative from Nueva Vizcaya. During the campaign of 1925 he resigned and ran for Representative of the Second District of Rizal, was elected and reelected in the following election, holding the position when he was appointed to the Cabinet.

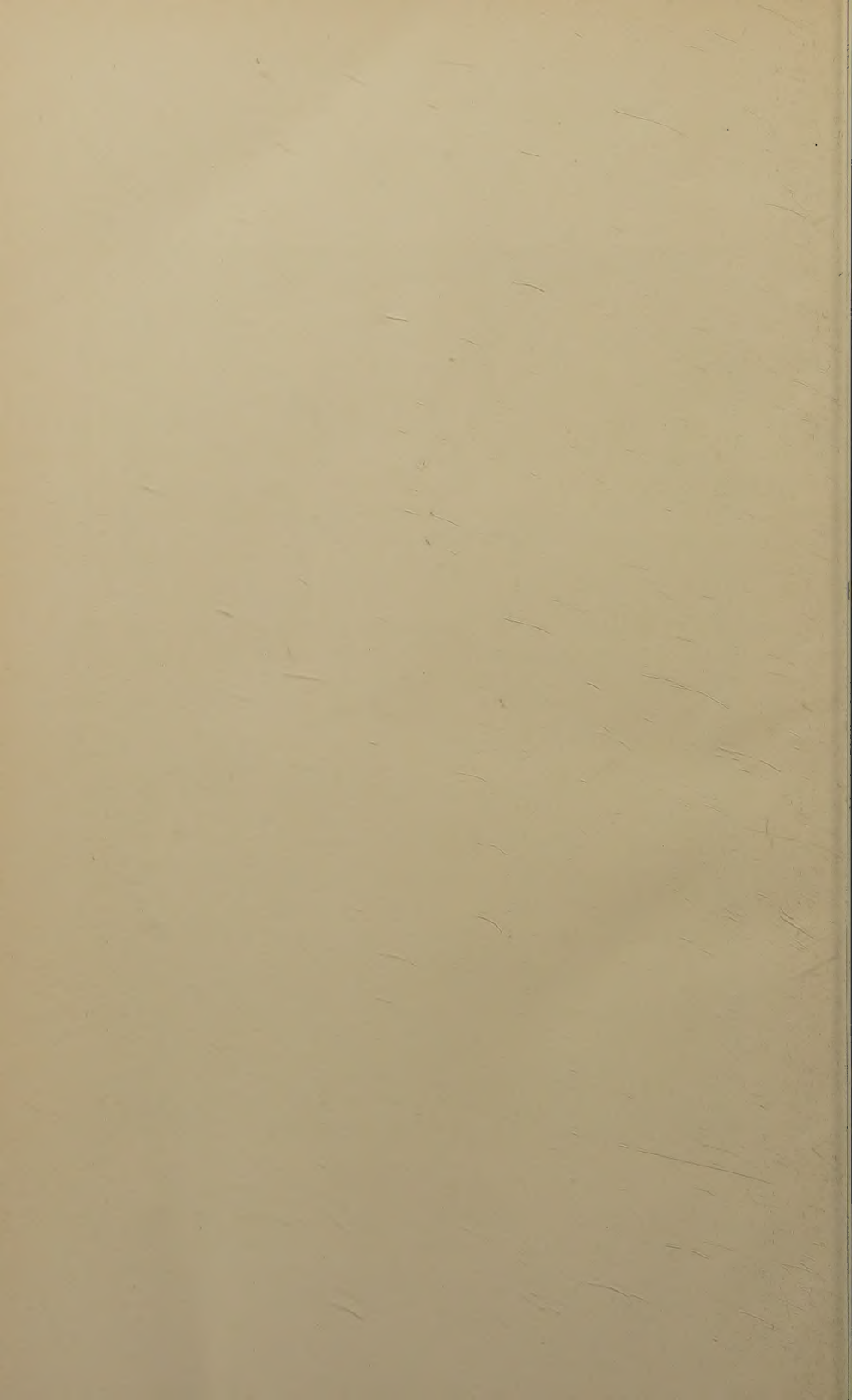
The appointment of Secretary Rodriguez to head the Department which is vested with the promotion of the economic development of the Philippines is hailed in all quarters as wise and well-deserved. The pressing problems to be faced by the coming Commonwealth Government are mainly economic. How to meet the loss of the American markets for the Philippine products and all the other factors incident thereto is partly explained by Secretary Rodriguez in a speech he recently delivered before the Rotary Club, and in which he expounded the policies he will pursue in meeting these problems. Among other things, Secretary Rodriguez said:

“The transitory period under the Commonwealth Government as provided for in the Tydings-McDuffie Law, is for economic readjustment. In going about our economic readjustment, in this particular phase, we should profit by our recent experience in the United States where exclusion of some of our important products of export is being pressed upon Congress. This experience should serve as a guide in the formulation of our policy for foreign trade expansion, bearing in mind the simple and rudimentary rule of conduct, upon which commercial relations among nations are based, that through foreign trade a country should secure only those commodities which it cannot produce advantageously, in exchange for its export of commodities that it can produce to greater advantage. \* \* \*

“The second statement I want to make is about our domestic trade. We have sadly neglected to develop our domestic trade. Due to excessive attention given to problems of our foreign markets, we have overlooked the



Secretary Eulogio Rodriguez (fifth on first row) and outgoing Secretary Vicente Singson Encarnacion (fourth on first row) with Directors of Bureaus and Chiefs of Divisions of the Department of Agriculture and Commerce.



importance of our own domestic market. While we need foreign demand, we must endeavour to strengthen our domestic market. Obviously, the domestic market is more stable than foreign markets, but the stability of the domestic market depends on the extent in which it is developed.

"Because land is man's great opportunity for the making of a living, the excessive abundance of land in our country have detracted our people from industrial pursuits. We have been following the natural course in our economic development, but in our task of economic readjustment we should set a detour in our orientation towards industrialization. This we can very well do, inasmuch as our abundant natural resources do not obstruct but facilitate our industrial advancement. \* \* \*

"My third statement, related to the second, is on the attainment of economic self-sufficiency for the Philippines, in the relative sense in which this term is generally accepted.

"In the formulation of commercial policies, two factors play a decisive part in their shaping: the economic strength of a country and its political situation. It is essential for any nation to have both political independence and economic independence. The first, so that it may shape intelligently, freely and without hindrance whatsoever the functions of government in a fashion that suits best its interests, and so that it may insure its economic development. Economic independence is likewise necessary, because it is the strength to guarantee political independence. The attainment of economic independence, in the generally accepted point of view of economists, may be possible by the development of varied industries using domestic raw materials and by the establishment of an unbreakable chain of commercial relations, which would give access to the ports and products of the trading world.

"In so far as the economic independence of the Philippines is concerned, it will not come to us if we try to isolate ourselves from the rest of the world, because this is against the principle of economic interdependence of nations. It will only come to us through the maximum development of our material resources. This includes not only the exploitation of our natural resources but industrial development as well. The demand of the consumers for products they need to satisfy their many requirements is not for raw materials but for manufactured articles. Unless, therefore, we have come to develop our industrial possibilities, we can not very well say that we are enjoying self-sufficiency."

E. R. A.

